

4

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

IN RE APPLICATION OF: Bettina MOECKEL et al

SERIAL NO: New U.S. Application

FILED: Herewith

FOR: NUCLEOTIDE SEQUENCES WHICH CODE FOR THE rpoB GENE

REQUEST FOR PRIORITY



ASSISTANT COMMISSIONER FOR PATENTS
WASHINGTON, D.C. 20231

SIR:

- ☐ Full benefit of the filing date of U.S. Application Serial Number, filed, is claimed pursuant to the provisions of 35 U.S.C. §120.
- ☐ Full benefit of the filing date of U.S. Provisional Application Serial Number, filed, is claimed pursuant to the provisions of 35 U.S.C. §119(e).
- ☒ Applicants claim any right to priority from any earlier filed applications to which they may be entitled pursuant to the provisions of 35 U.S.C. §119, as noted below.

In the matter of the above-identified application for patent, notice is hereby given that the applicants claim as priority:

<u>COUNTRY</u>	<u>APPLICATION NUMBER</u>	<u>MONTH/DAY/YEAR</u>
GERMANY	101 01 229.5	February 16, 2001

Certified copies of the corresponding Convention Application(s)

- ☒ is submitted herewith
- ☐ will be submitted prior to payment of the Final Fee
- ☐ were filed in prior application Serial No. filed
- ☐ were submitted to the International Bureau in PCT Application Number .
Receipt of the certified copies by the International Bureau in a timely manner under PCT Rule 17.1(a) has been acknowledged as evidenced by the attached PCT/IB/304.
- ☐ (A) Application Serial No.(s) were filed in prior application Serial No. filed ; and
(B) Application Serial No.(s)
 - ☐ are submitted herewith
 - ☐ will be submitted prior to payment of the Final Fee

Respectfully Submitted,

OBLON, SPIVAK, McCLELLAND,
MAIER & NEUSTADT, P.C.

Norman F. Oblon
Registration No. 24,618

Daniel J. Pereira, Ph.D.
Registration No. 45,518



22850



Prioritätsbescheinigung über die Einreichung einer Patentanmeldung

Aktenzeichen: 101 07 229.5

Anmeldetag: 16. Februar 2001

Anmelder/Inhaber: Degussa AG, Düsseldorf/DE

Bezeichnung: Neue für das rpoB-Gen kodierende Nukleotidsequenzen

IPC: C 12 N, C 12 Q, C 07 H

Bemerkung: Die nachgereichte Seite 39 der Beschreibung ist am 28. April 2001 eingegangen.

Die angehefteten Stücke sind eine richtige und genaue Wiedergabe der ursprünglichen Unterlagen dieser Patentanmeldung.

München, den 29. Mai 2001
Deutsches Patent- und Markenamt
Der Präsident
Im Auftrag

Hiebinger

Neue für das rpoB-Gen kodierende Nukleotidsequenzen

Gegenstand der Erfindung sind für das rpoB-Gen kodierende Nukleotidsequenzen aus coryneformen Bakterien und ein Verfahren zur fermentativen Herstellung von Aminosäuren unter Verwendung von Bakterien, in denen das rpoB-Gen

5 verstärkt wird.

Stand der Technik

L-Aminosäuren, insbesondere L-Lysin, finden in der Humanmedizin und in der pharmazeutischen Industrie, in der Lebensmittelindustrie und ganz besonders in der

10 Tierernährung, Anwendung.

Es ist bekannt, daß Aminosäuren durch Fermentation von Stämmen coryneformer Bakterien, insbesondere Corynebacterium glutamicum, hergestellt werden. Wegen der

15 großen Bedeutung wird ständig an der Verbesserung der Herstellverfahren gearbeitet. Verfahrensverbesserungen können fermentationstechnische Maßnahmen wie zum Beispiel Rührung und Versorgung mit Sauerstoff, oder die Zusammensetzung der Nährmedien wie zum Beispiel die

20 Zuckerkonzentration während der Fermentation, oder die Aufarbeitung zur Produktform durch zum Beispiel Ionenaustauschchromatographie oder die intrinsischen Leistungseigenschaften des Mikroorganismus selbst betreffen.

25 Zur Verbesserung der Leistungseigenschaften dieser Mikroorganismen werden Methoden der Mutagenese, Selektion und Mutantenauswahl angewendet. Auf diese Weise erhält man Stämme, die resistent gegen Antimetabolite oder auxotroph für regulatorisch bedeutsame Metabolite sind und

30 Aminosäuren produzieren.

Seit einigen Jahren werden ebenfalls Methoden der rekombinanten DNA-Technik zur Stammverbesserung von

L-Aminosäure produzierenden Stämmen von *Corynebacterium* eingesetzt, indem man einzelne Aminosäure-Biosynthesegene amplifiziert und die Auswirkung auf die Aminosäure-Produktion untersucht.

5 Aufgabe der Erfindung

Die Erfinder haben sich zur Aufgabe gestellt, neue Maßnahmen zur verbesserten fermentativen Herstellung von Aminosäuren bereitzustellen.

Beschreibung der Erfindung

- 10 Werden im folgenden L-Aminosäuren oder Aminosäuren erwähnt, sind damit eine oder mehrere Aminosäuren einschließlich ihrer Salze, ausgewählt aus der Gruppe L-Asparagin, L-Threonin, L-Serin, L-Glutamat, L-Glycin, L-Alanin, L-Cystein, L-Valin, L-Methionin, L-Isoleucin, L-Leucin, L-Tyrosin, L-Phenylalanin, L-Histidin, L-Lysin, L-Tryptophan und L-Arginin gemeint. Besonders bevorzugt ist L-Lysin.

Wenn im folgenden L-Lysin oder Lysin erwähnt werden, sind damit nicht nur die Basen, sondern auch die Salze wie z.B. Lysin-Monohydrochlorid oder Lysin-Sulfat gemeint.

- 20 Gegenstand der Erfindung ist ein isoliertes Polynukleotid aus coryneformen Bakterien, enthaltend eine für das rpoB-Gen kodierende Polynukleotidsequenz, ausgewählt aus der Gruppe

- a) Polynukleotid, das mindestens zu 70% identisch ist mit
25 einem Polynukleotid, das für ein Polypeptid kodiert, das die Aminosäuresequenz von SEQ ID No. 2 enthält,
- b) Polynukleotid, das für ein Polypeptid kodiert, das eine Aminosäuresequenz enthält, die zu mindestens 70% identisch ist mit der Aminosäuresequenz von
30 SEQ ID No. 2,

c) Polynukleotid, das komplementär ist zu den Polynukleotiden von a) oder b), und

5 d) Polynukleotid, enthaltend mindestens 15 aufeinanderfolgende Nukleotide der Polynukleotidsequenz von a), b) oder c),

wobei das Polypeptid bevorzugt die Aktivität der β -Untereinheit der RNA-Polymerase B aufweist.

10 Gegenstand der Erfindung ist ebenfalls das oben genannte Polynukleotid, wobei es sich bevorzugt um eine replizierbare DNA handelt, enthaltend:

- (i) die Nukleotidsequenz, gezeigt in SEQ ID No. 1, oder
- 15 (ii) mindestens eine Sequenz, die der Sequenz (i) innerhalb des Bereichs der Degeneration des genetischen Kodes entspricht, oder
- (iii) mindestens eine Sequenz, die mit der zur Sequenz (i) oder (ii) komplementären Sequenz hybridisiert, und gegebenenfalls
- 20 (iv) funktionsneutralen Sinnmutationen in (i), die die Aktivität des Proteins/Polypeptides nicht verändern

Ein weiterer Gegenstand der Erfindung sind schließlich Polynukleotide ausgewählt aus der Gruppe

- 25 a) Polynukleotide enthaltend mindestens 15 aufeinanderfolgende Nukleotide ausgewählt aus der Nukleotidsequenz von SEQ ID No. 1 zwischen den Positionen 1 und 701
- b) Polynukleotide enthaltend mindestens 15 aufeinanderfolgende Nukleotide ausgewählt aus der

Nukleotidsequenz von SEQ ID No. 1 zwischen den Positionen 702 und 4199

- 5 c) Polynukleotide enthaltend mindestens 15 aufeinanderfolgende Nukleotide ausgewählt aus der Nukleotidsequenz von SEQ ID No. 1 zwischen den Positionen 4200 und 5099.

Weitere Gegenstände sind

10 ein replizierbares Polynukleotid, insbesondere DNA, enthaltend die Nukleotidsequenz wie in SEQ ID No. 1 dargestellt;

ein Polynukleotid, das für ein Polypeptid kodiert, das die Aminosäuresequenz, wie in SEQ ID No. 2 dargestellt, enthält;

15 ein Vektor, enthaltend das erfindungsgemäße Polynukleotid, insbesondere Pendelvektor oder Plasmidvektor, und

coryneforme Bakterien, die den Vektor enthalten oder in denen das rpoB-Gen verstärkt ist.

20 Gegenstand der Erfindung sind ebenso Polynukleotide, die im wesentlichen aus einer Polynukleotidsequenz bestehen, die erhältlich sind durch Screening mittels Hybridisierung einer entsprechenden Genbank eines coryneformen Bakteriums, die das vollständige Gen oder Teile davon enthält, mit einer Sonde, die die Sequenz des erfindungsgemäßen Polynukleotids gemäß SEQ ID No.1 oder ein Fragment davon
25 enthält und Isolierung der genannten Polynukleotidsequenz.

Polynukleotide, die die Sequenzen gemäß der Erfindung enthalten, sind als Hybridisierungs-Sonden für RNA, cDNA und DNA geeignet, um Nukleinsäuren beziehungsweise Polynukleotide oder Gene in voller Länge zu isolieren, die
30 für die β -Untereinheit der RNA-Polymerase B kodieren, oder um solche Nukleinsäuren beziehungsweise Polynukleotide oder

Gene zu isolieren, die eine hohe Ähnlichkeit der Sequenz mit der des rpoB-Gens aufweisen. Sie sind ebenso zum Einbau in sogenannte „arrays“, „micro arrays“ oder „DNA chips“ geeignet, um die entsprechenden Polynukleotide zu
5 detektieren und zu bestimmen

Polynukleotide, die die Sequenzen gemäß der Erfindung enthalten, sind weiterhin als Primer geeignet, mit deren Hilfe mit der Polymerase-Kettenreaktion (PCR) DNA von Genen hergestellt werden kann, die für die β -Untereinheit der
10 RNA-Polymerase B kodieren.

Solche als Sonden oder Primer dienende Oligonukleotide, enthalten mindestens 25, 26, 27, 28, 29 oder 30, bevorzugt mindestens 20, 21, 22, 23 oder 24, ganz besonders bevorzugt mindestens 15, 16, 17, 18 oder 19 aufeinanderfolgende
15 Nukleotide. Geeignet sind ebenfalls Oligonukleotide mit einer Länge von mindestens 31, 32, 33, 34, 35, 36, 37, 38, 39 oder 40, oder mindestens 41, 42, 43, 44, 45, 46, 47, 48, 49 oder 50 Nukleotiden. Gegebenenfalls sind auch Oligonukleotide mit einer Länge von mindestens 100, 150,
20 200, 250 oder 300 Nukleotiden geeignet.

„Isoliert“ bedeutet aus seinem natürlichen Umfeld herausgetrennt.

„Polynukleotid“ bezieht sich im allgemeinen auf Polyribonukleotide und Polydeoxyribonukleotide, wobei es
25 sich um nicht modifizierte RNA oder DNA oder modifizierte RNA oder DNA handeln kann.

Die Polynukleotide gemäß Erfindung schließen ein Polynukleotid gemäß SEQ ID No. 1 oder ein daraus hergestelltes Fragment und auch solche ein, die zu
30 wenigstens besonders 70% bis 80%, bevorzugt zu wenigstens 81% bis 85%, besonders bevorzugt zu wenigstens 86% bis 90%, und ganz besonders bevorzugt zu wenigstens 91%, 93%, 95%,

97% oder 99% identisch sind mit dem Polynukleotid gemäß SEQ ID No. 1 oder eines daraus hergestellten Fragmentes.

Unter „Polypeptiden“ versteht man Peptide oder Proteine, die zwei oder mehr über Peptidbindungen verbundene
5 Aminosäuren enthalten.

Die Polypeptide gemäß Erfindung schließen ein Polypeptid gemäß SEQ ID No. 2, insbesondere solche mit der biologischen Aktivität der β -Untereinheit der RNA-Polymerase B und auch solche ein, die zu wenigstens 70% bis
10 80%, bevorzugt zu wenigstens 81% bis 85%, besonders bevorzugt zu wenigstens 86% bis 90%, und ganz besonders bevorzugt zu wenigstens 91%, 93%, 95%, 97% oder 99% identisch sind mit dem Polypeptid gemäß SEQ ID No. 2 und die genannte Aktivität aufweisen.

15 Die Erfindung betrifft weiterhin ein Verfahren zur fermentativen Herstellung von Aminosäuren, ausgewählt aus der Gruppe L-Asparagin, L-Threonin, L-Serin, L-Glutamat, L-Glycin, L-Alanin, L-Cystein, L-Valin, L-Methionin, L-Isoleucin, L-Leucin, L-Tyrosin, L-
20 Phenylalanin, L-Histidin, L-Lysin, L-Tryptophan und L-Arginin, unter Verwendung von coryneformen Bakterien, die insbesondere bereits Aminosäuren produzieren und in denen die für das rpoB-Gen kodierenden Nukleotidsequenzen verstärkt, insbesondere überexprimiert werden.

25 Der Begriff „Verstärkung“ beschreibt in diesem Zusammenhang die Erhöhung der intrazellulären Aktivität eines oder mehrerer Enzyme bzw. Proteine in einem Mikroorganismus, die durch die entsprechende DNA kodiert werden, indem man beispielsweise die Kopienzahl des Gens bzw. der Gene
30 erhöht, einen starken Promotor verwendet oder ein Gen oder Allel verwendet, das für ein entsprechendes Enzym bzw. Protein mit einer hohen Aktivität kodiert und gegebenenfalls diese Maßnahmen kombiniert.

Die Mikroorganismen, die Gegenstand der vorliegenden Erfindung sind, können L-Aminosäuren aus Glucose, Saccharose, Lactose, Fructose, Maltose, Melasse, Stärke, Cellulose oder aus Glycerin und Ethanol herstellen. Es kann
5 sich um Vertreter coryneformer Bakterien insbesondere der Gattung *Corynebacterium* handeln. Bei der Gattung *Corynebacterium* ist insbesondere die Art *Corynebacterium glutamicum* zu nennen, die in der Fachwelt für ihre Fähigkeit bekannt ist, L-Aminosäuren zu produzieren.

10 Geeignete Stämme der Gattung *Corynebacterium*, insbesondere der Art *Corynebacterium glutamicum* (*C. glutamicum*), sind besonders die bekannten Wildtypstämme

Corynebacterium glutamicum ATCC13032
Corynebacterium acetoglutamicum ATCC15806
15 *Corynebacterium acetoacidophilum* ATCC13870
Corynebacterium thermoaminogenes FERM BP-1539
Corynebacterium melassecola ATCC17965
Brevibacterium flavum ATCC14067
Brevibacterium lactofermentum ATCC13869 und
20 *Brevibacterium divaricatum* ATCC14020

und daraus hergestellte L-Aminosäuren produzierende Mutanten bzw. Stämme, wie beispielsweise die L-Lysin produzierenden Stämme

Corynebacterium glutamicum FERM-P 1709
25 *Brevibacterium flavum* FERM-P 1708
Brevibacterium lactofermentum FERM-P 1712
Corynebacterium glutamicum FERM-P 6463
Corynebacterium glutamicum FERM-P 6464
Corynebacterium glutamicum DM58-1
30 *Corynebacterium glutamicum* DG52-5
Corynebacterium glutamicum DSM5714 und
Corynebacterium glutamicum DSM12866.

Das neue, für die β -Untereinheit der RNA-Polymerase B kodierende rpoB-Gen von *C. glutamicum* wurde isoliert.

- Zur Isolierung des rpoB-Gens oder auch anderer Gene von *C. glutamicum* wird zunächst eine Genbank dieses
- 5 Mikroorganismus in *Escherichia coli* (*E. coli*) angelegt. Das Anlegen von Genbanken ist in allgemein bekannten Lehrbüchern und Handbüchern niedergeschrieben. Als Beispiel seien das Lehrbuch von Winnacker: Gene und Klone, Eine
- 10 Einführung in die Gentechnologie (Verlag Chemie, Weinheim, Deutschland, 1990), oder das Handbuch von Sambrook et al.: Molecular Cloning, A Laboratory Manual (Cold Spring Harbor Laboratory Press, 1989) genannt. Eine sehr bekannte Genbank ist die des *E. coli* K-12 Stammes W3110, die von Kohara et al. (Cell 50, 495-508 (1987)) in λ -Vektoren angelegt wurde.
- 15 Bathe et al. (Molecular and General Genetics, 252:255-265, 1996) beschreiben eine Genbank von *C. glutamicum* ATCC13032, die mit Hilfe des Cosmidvektors SuperCos I (Wahl et al., 1987, Proceedings of the National Academy of Sciences USA, 84:2160-2164) im *E. coli* K-12 Stamm NM554 (Raleigh et al.,
- 20 1988, Nucleic Acids Research 16:1563-1575) angelegt wurde.

Börmann et al. (Molecular Microbiology 6(3), 317-326 (1992)) wiederum beschreiben eine Genbank von *C. glutamicum* ATCC13032 unter Verwendung des Cosmids pH79 (Hohn und Collins, Gene 11, 291-298 (1980)).

- 25 Zur Herstellung einer Genbank von *C. glutamicum* in *E. coli* können auch Plasmide wie pBR322 (Bolivar, Life Sciences, 25, 807-818 (1979)) oder pUC9 (Vieira et al., 1982, Gene, 19:259-268) verwendet werden. Als Wirte eignen sich besonders solche *E. coli* Stämme, die restriktions- und
- 30 rekombinationsdefekt sind. Ein Beispiel hierfür ist der Stamm DH5 α mc^r, der von Grant et al. (Proceedings of the National Academy of Sciences USA, 87 (1990) 4645-4649) beschrieben wurde. Die mit Hilfe von Cosmiden klonierten langen DNA-Fragmente können anschließend wiederum in
- 35 gängige, für die Sequenzierung geeignete Vektoren

subkloniert und anschließend sequenziert werden, so wie es z.B. bei Sanger et al. (Proceedings of the National Academy of Sciences of the United States of America, 74:5463-5467, 1977) beschrieben ist.

- 5 Die erhaltenen DNA-Sequenzen können dann mit bekannten Algorithmen bzw. Sequenzanalyse-Programmen wie z.B. dem von Staden (Nucleic Acids Research 14, 217-232(1986)), dem von Marck (Nucleic Acids Research 16, 1829-1836 (1988)) oder dem GCG-Programm von Butler (Methods of Biochemical
10 Analysis 39, 74-97 (1998)) untersucht werden.

- Die neue für das Gen *rpoB* kodierende DNA-Sequenz von *C. glutamicum* wurde gefunden, die als SEQ ID No. 1 Bestandteil der vorliegenden Erfindung ist. Weiterhin wurde aus der vorliegenden DNA-Sequenz mit den oben beschriebenen
15 Methoden die Aminosäuresequenz des entsprechenden Proteins abgeleitet. In SEQ ID No. 2 ist die sich ergebende Aminosäuresequenz des *rpoB*-Genproduktes dargestellt. Es ist bekannt, daß wirtseigene Enzyme die N-terminale Aminosäure Methionin bzw. Formylmethionin des gebildeten Proteins
20 abspalten können.

- Kodierende DNA-Sequenzen, die sich aus SEQ ID No. 1 durch die Degeneriertheit des genetischen Kodes ergeben, sind ebenfalls Bestandteil der Erfindung. In gleicher Weise sind DNA-Sequenzen, die mit SEQ ID No. 1 oder Teilen von SEQ ID
25 No. 1 hybridisieren, Bestandteil der Erfindung. In der Fachwelt sind weiterhin konservative Aminosäureaustausche wie z.B. Austausch von Glycin gegen Alanin oder von Asparaginsäure gegen Glutaminsäure in Proteinen als „Sinnmutationen“ („sense mutations“) bekannt, die zu keiner
30 grundsätzlichen Veränderung der Aktivität des Proteins führen, d.h. funktionsneutral sind. Derartige Mutationen werden unter anderem auch als neutrale Substitutionen bezeichnet. Weiterhin ist bekannt, daß Änderungen am N- und/oder C-Terminus eines Proteins dessen Funktion nicht
35 wesentlich beeinträchtigen oder sogar stabilisieren können.

Angaben hierzu findet der Fachmann unter anderem bei Ben-Bassat et al. (Journal of Bacteriology 169:751-757 (1987)), bei O'Regan et al. (Gene 77:237-251 (1989)), bei Sahin-Toth et al. (Protein Sciences 3:240-247 (1994)), bei Hochuli et al. (Bio/Technology 6:1321-1325 (1988)) und in bekannten Lehrbüchern der Genetik und Molekularbiologie.

Aminosäuresequenzen, die sich in entsprechender Weise aus SEQ ID No. 2 ergeben, sind ebenfalls Bestandteil der Erfindung.

10 In gleicher Weise sind DNA-Sequenzen, die mit SEQ ID No. 1 oder Teilen von SEQ ID No. 1 hybridisieren Bestandteil der Erfindung. Schließlich sind DNA-Sequenzen Bestandteil der Erfindung, die durch die Polymerase-Kettenreaktion (PCR) unter Verwendung von Primern hergestellt werden, die sich
15 aus SEQ ID No. 1 ergeben. Derartige Oligonukleotide haben typischerweise eine Länge von mindestens 15 Nukleotiden.

Anleitungen zur Identifizierung von DNA-Sequenzen mittels Hybridisierung findet der Fachmann unter anderem im Handbuch "The DIG System Users Guide for Filter
20 Hybridization" der Firma Boehringer Mannheim GmbH (Mannheim, Deutschland, 1993) und bei Liebl et al. (International Journal of Systematic Bacteriology (1991) 41: 255-260). Die Hybridisierung findet unter stringenten Bedingungen statt, das heisst, es werden nur Hybride
25 gebildet, bei denen Sonde und Zielsequenz, d. h. die mit der Sonde behandelten Polynukleotide, mindestens 70% identisch sind. Es ist bekannt, dass die Stringenz der Hybridisierung einschließlich der Waschschrirte durch Variieren der Pufferzusammensetzung, der Temperatur und der
30 Salzkonzentration beeinflußt bzw. bestimmt wird. Die Hybridisierungsreaktion wird vorzugsweise bei relativ niedriger Stringenz im Vergleich zu den Waschschrirten durchgeführt (Hybaid Hybridisation Guide, Hybaid Limited, Teddington, UK, 1996).

- Für die Hybridisierungsreaktion kann beispielsweise ein 5x SSC-Puffer bei einer Temperatur von ca. 50°C - 68°C eingesetzt werden. Dabei können Sonden auch mit Polynukleotiden hybridisieren, die weniger als 70%
- 5 Identität zur Sequenz der Sonde aufweisen. Solche Hybride sind weniger stabil und werden durch Waschen unter stringenten Bedingungen entfernt. Dies kann beispielsweise durch Senken der Salzkonzentration auf 2x SSC und gegebenenfalls nachfolgend 0,5x SSC (The DIG System User's
- 10 Guide for Filter Hybridisation, Boehringer Mannheim, Mannheim, Deutschland, 1995) erreicht werden, wobei eine Temperatur von ca. 50°C - 68°C eingestellt wird. Es ist gegebenenfalls möglich die Salzkonzentration bis auf 0,1x SSC zu senken. Durch schrittweise Erhöhung der
- 15 Hybridisierungstemperatur in Schritten von ca. 1 - 2°C von 50°C auf 68°C können Polynukleotidfragmente isoliert werden, die beispielsweise mindestens 70% oder mindestens 80% oder mindestens 90% bis 95% Identität zur Sequenz der eingesetzten Sonde besitzen. Weitere Anleitungen zur
- 20 Hybridisierung sind in Form sogenannter Kits am Markt erhältlich (z.B. DIG Easy Hyb von der Firma Roche Diagnostics GmbH, Mannheim, Deutschland, Catalog No. 1603558).

- 25 Anleitungen zur Amplifikation von DNA-Sequenzen mit Hilfe der Polymerase-Kettenreaktion (PCR) findet der Fachmann unter anderem im Handbuch von Gait: Oligonukleotide synthesis: A Practical Approach (IRL Press, Oxford, UK, 1984) und bei Newton und Graham: PCR (Spektrum Akademischer Verlag, Heidelberg, Deutschland, 1994).

- 30 Es wurde gefunden, daß coryneforme Bakterien nach Verstärkung des rpoB-Gens in verbesserter Weise Aminosäuren produzieren.

Zur Erzielung einer Überexpression kann die Kopienzahl der entsprechenden Gene erhöht werden, oder es kann die

Promotor- und Regulationsregion oder die Ribosomenbindungsstelle, die sich stromaufwärts des Strukturgens befindet, mutiert werden. In gleicher Weise wirken Expressionskassetten, die stromaufwärts des Strukturgens eingebaut werden. Durch induzierbare Promotoren ist es zusätzlich möglich, die Expression im Verlaufe der fermentativen Aminosäure-Produktion zu steigern. Durch Maßnahmen zur Verlängerung der Lebensdauer der m-RNA wird ebenfalls die Expression verbessert.

Weiterhin wird durch Verhinderung des Abbaus des Enzymproteins ebenfalls die Enzymaktivität verstärkt. Die Gene oder Genkonstrukte können entweder in Plasmiden mit unterschiedlicher Kopienzahl vorliegen oder im Chromosom integriert und amplifiziert sein. Alternativ kann weiterhin eine Überexpression der betreffenden Gene durch Veränderung der Medienzusammensetzung und Kulturführung erreicht werden.

Anleitungen hierzu findet der Fachmann unter anderem bei Martin et al. (Bio/Technology 5, 137-146 (1987)), bei Guerrero et al. (Gene 138, 35-41 (1994)), Tsuchiya und Morinaga (Bio/Technology 6, 428-430 (1988)), bei Eikmanns et al. (Gene 102, 93-98 (1991)), in der Europäischen Patentschrift 0 472 869, im US Patent 4,601,893, bei Schwarzer und Pühler (Bio/Technology 9, 84-87 (1991)), bei Reinscheid et al. (Applied and Environmental Microbiology 60, 126-132 (1994)), bei LaBarre et al. (Journal of Bacteriology 175, 1001-1007 (1993)), in der Patentanmeldung WO 96/15246, bei Malumbres et al. (Gene 134, 15 - 24 (1993)), in der japanischen Offenlegungsschrift JP-A-10-229891, bei Jensen und Hammer (Biotechnology and Bioengineering 58, 191-195 (1998)), bei Makrides (Microbiological Reviews 60:512-538 (1996)) und in bekannten Lehrbüchern der Genetik und Molekularbiologie.

Zur Verstärkung wurde das erfindungsgemäße rpoB-Gen beispielhaft mit Hilfe von episomalen Plasmiden

überexprimiert. Als Plasmide eignen sich solche, die in coryneformen Bakterien repliziert werden. Zahlreiche bekannte Plasmidvektoren wie z.B. pZ1 (Menkel et al., Applied and Environmental Microbiology (1989) 64: 549-554), pEKEx1 (Eikmanns et al., Gene 102:93-98 (1991)) oder pHS2-1 (Sonnen et al., Gene 107:69-74 (1991)) beruhen auf den kryptischen Plasmiden pHM1519, pBL1 oder pGA1. Andere Plasmidvektoren wie z.B. solche, die auf pCG4 (US-A 4,489,160), oder pNG2 (Serwold-Davis et al., FEMS Microbiology Letters 66, 119-124 (1990)), oder pAG1 (US-A 5,158,891) beruhen, können in gleicher Weise verwendet werden.

Weiterhin eignen sich auch solche Plasmidvektoren mit Hilfe derer man das Verfahren der Genamplifikation durch Integration in das Chromosom anwenden kann, so wie es beispielsweise von Reinscheid et al. (Applied and Environmental Microbiology 60, 126-132 (1994)) zur Duplikation bzw. Amplifikation des *hom-thrB*-Operons beschrieben wurde. Bei dieser Methode wird das vollständige Gen in einen Plasmidvektor kloniert, der in einem Wirt (typischerweise *E. coli*), nicht aber in *C. glutamicum* replizieren kann. Als Vektoren kommen beispielsweise pSUP301 (Simon et al., Bio/Technology 1, 784-791 (1983)), pK18mob oder pK19mob (Schäfer et al., Gene 145, 69-73 (1994)), pGEM-T (Promega corporation, Madison, WI, USA), pCR2.1-TOPO (Shuman (1994). Journal of Biological Chemistry 269:32678-84; US-A 5,487,993), pCR®Blunt (Firma Invitrogen, Groningen, Niederlande; Bernard et al., Journal of Molecular Biology, 234: 534-541 (1993)), pEM1 (Schrumpf et al, 1991, Journal of Bacteriology 173:4510-4516) oder pBGS8 (Spratt et al., 1986, Gene 41: 337-342) in Frage. Der Plasmidvektor, der das zu amplifizierende Gen enthält, wird anschließend durch Konjugation oder Transformation in den gewünschten Stamm von *C. glutamicum* überführt. Die Methode der Konjugation ist beispielsweise bei Schäfer et al. (Applied and Environmental Microbiology 60, 756-759 (1994))

beschrieben. Methoden zur Transformation sind beispielsweise bei Thierbach et al. (Applied Microbiology and Biotechnology 29, 356-362 (1988)), Dunican und Shivnan (Bio/Technology 7, 1067-1070 (1989)) und Tauch et al. (FEMS Microbiological Letters 123, 343-347 (1994)) beschrieben. Nach homologer Rekombination mittels eines "cross over"-Ereignisses enthält der resultierende Stamm mindestens zwei Kopien des betreffenden Gens.

Es wurde weiterhin gefunden, dass der Austausch von Aminosäuren, insbesondere in den Abschnitten zwischen Position 1 bis 10, 190 bis 200 und 420 bis 450 in der Aminosäuresequenz der β -Untereinheit der RNA-Polymerase B dargestellt in SEQ ID No. 2, die Lysinproduktion coryneformer Bakterien verbessern.

Es wurde auch gefunden, dass der Austausch von Aminosäuren an einer oder mehreren Positionen ausgewählt aus der Gruppe a) Position 1 bis 10, b) Position 190 bis 200 und c) Position 420 bis 450 in der SEQ ID No. 2 gleichzeitig erfolgen kann.

In dem Bereich zwischen Position 1 bis 10 wird der Austausch von L-Prolin an der Position 5 gegen L-Leucin, L-Isoleucin oder L-Valin bevorzugt.

In dem Bereich zwischen Position 190 bis 200 wird der Austausch von L-Serin an der Position 196 gegen L-Phenylalanin oder L-Tyrosin bevorzugt.

In dem Bereich zwischen 420 bis 450 werden folgende Austausche bevorzugt: Austausch von L-Leucin an der Position 424 gegen L-Prolin oder L-Arginin, Austausch von L-Serin an der Position 425 gegen L-Threonin oder L-Alanin, Austausch von L-Glutamin an Position 426 gegen L-Leucin oder L-Lysin, Austausch von L-Asparaginsäure an Position 429 gegen L-Isoleucin, L-Valin oder L-Leucin, Austausch von L-Histidin an Position 439 gegen jede andere proteinogene

Aminosäure ausgenommen L-Histidin, wird der Austausch von L-Serin an Position 444 gegen L-Leucin, L-Tyrosin oder L-Tryptophan und Austausch von L-Leucin an Position 446 gegen L-Prolin oder L-Isoleucin.

- 5 Ganz besonders bevorzugt, werden ein oder mehrere Aminosäureaustausche ausgewählt aus der Gruppe: L-Prolin an Position 5 gegen L-Leucin, L-Serin an Position 196 gegen L-Phenylalanin, L-Aspartat an Position 429 gegen L-Valin und L-Histidin an Position 439 gegen L-Tyrosin.
- 10 In SEQ ID No. 3 ist die Basensequenz des in Stamm DM1547 enthaltenen Allels rpoB-1547 dargestellt. Das rpoB-1547 Allel kodiert für ein Protein, dessen Aminosäuresequenz in SEQ ID No. 4 dargestellt ist. Das Protein enthält an Position 5 L-Leucin, an Position 196 L-Phenylalanin und an Position 429 L-Valin. Die DNA Sequenz des rpoB-1547 Allels (SEQ ID No. 3) enthält folgende Basenaustausche gegenüber dem rpoB Wildtypgen (SEQ ID No. 1): Thymin an Position 715 anstelle von Cytosin, Thymin an Position 1288 anstelle von Cytosin und Thymin an Position 1987 anstelle von Adenin.
- 20 In SEQ ID No. 5 ist die Basensequenz des in Stamm DM1546 enthaltenen Allels rpoB-1546 dargestellt. Das rpoB-1546 Allel kodiert für ein Protein, dessen Aminosäuresequenz in SEQ ID No. 6 dargestellt ist. Das Protein enthält an Position 439 L-Tyrosin. Die DNA Sequenz des rpoB-1546 Allels (SEQ ID No. 5) enthält folgende Basenaustausche gegenüber dem rpoB Wildtypgen (SEQ ID No. 1): Thymin an Position 2016 anstelle von Cytosin.

- Für die Mutagenese können klassische Mutageneseverfahren unter Verwendung mutagener Stoffe wie beispielsweise N-Methyl-N'-Nitro-N-Nitrosoguanidin oder ultraviolettes Licht verwendet werden. Weiterhin können für die Mutagenese in-vitro Methoden wie beispielsweise eine Behandlung mit Hydroxylamin (Miller, J. H.: A Short Course in Bacterial Genetics. A Laboratory Manual and Handbook for Escherichia
- 30

coli and Related Bacteria, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, 1992) oder mutagene Oligonukleotide (T. A. Brown: Gentechnologie für Einsteiger, Spektrum Akademischer Verlag, Heidelberg, 1993) oder die Polymerasekettenreaktion (PCR), wie sie im Handbuch von Newton und Graham (PCR, Spektrum Akademischer Verlag, Heidelberg, 1994) beschrieben ist, verwendet werden.

10 Zusätzlich kann es für die Produktion von L-Aminosäuren vorteilhaft sein, neben dem rpoB-Gen eines oder mehrere Enzyme des jeweiligen Biosyntheseweges, der Glykolyse, der Anaplerotik, des Zitronensäure-Zyklus, des Pentosephosphat-Zyklus, des Aminosäure-Exports und gegebenenfalls regulatorische Proteine zu verstärken, insbesondere
15 überzuexprimieren.

So kann für die Herstellung von L-Lysin zusätzlich zur Verstärkung des rpoB-Gens eines oder mehrere der Gene, ausgewählt aus der Gruppe

- 20 • das für die Dihydrodipicolinat-Synthase kodierende Gen dapA (EP-B 0 197 335),
- das für die Glyceraldehyd-3-Phosphat-Dehydrogenase kodierende Gen gap (Eikmanns (1992), Journal of Bacteriology 174:6076-6086),
- 25 • das für die Triosephosphat-Isomerase kodierende Gen tpi (Eikmanns (1992), Journal of Bacteriology 174:6076-6086),
- das für die 3-Phosphoglycerat-Kinase kodierende Gen pgk (Eikmanns (1992), Journal of Bacteriology 174:6076-6086),
- das für die Glucose-6-Phosphat-Dehydrogenase kodierende Gen zwf (JP-A-09224661),
- 30 • das für die Pyruvat-Carboxylase kodierende Gen pyc (DE-A-198 31 609),

- das für die Malat-Chinon-Oxidoreduktase kodierende Gen mgo (Molenaar et al., European Journal of Biochemistry 254, 395-403 (1998)),
- 5 • das für eine feed-back resistente Aspartatkinase kodierende Gen lysC (Kalinowski et al., Molecular Microbiologie 5(5), 1197-204 (1991)),
- das für den Lysin-Export kodierende Gen lysE (DE-A-195 48 222),
- 10 • das für das Zwa1-Protein kodierende Gen zwa1 (DE: 19959328.0, DSM 13115), und
- das für das ribosomale Protein S12 kodierende rpsL-Gen dargestellt in SEQ ID No. 7 und 8

verstärkt, insbesondere überexprimiert werden.

Der Begriff „Abschwächung“ beschreibt in diesem
15 Zusammenhang die Verringerung oder Ausschaltung der intrazellulären Aktivität eines oder mehrerer Enzyme (Proteine) in einem Mikroorganismus, die durch die entsprechende DNA kodiert werden, indem man beispielsweise einen schwachen Promotor verwendet oder ein Gen bzw. Allel
20 verwendet, das für ein entsprechendes Enzym mit einer niedrigen Aktivität kodiert bzw. das entsprechende Gen oder Enzym (Protein) inaktiviert und gegebenenfalls diese Maßnahmen kombiniert.

Weiterhin kann es für die Produktion von L-Aminosäuren
25 vorteilhaft sein, zusätzlich zur Verstärkung des rpoB-Gens eines oder mehrere Gene, ausgewählt aus der Gruppe

- das für die Phosphoenolpyruvat-Carboxykinase kodierende Gen pck (DE 199 50 409.1; DSM 13047),
- 30 • das für die Glucose-6-Phosphat-Isomerase kodierende Gen pgi (US 09/396,478; DSM 12969),

- das für die Pyruvat-Oxidase kodierende Gen *poxB* (DE: 1995 1975.7; DSM 13114),
- das für das Zwa2-Protein kodierende Gen *zwa2* (DE: 19959327.2, DSM 13113)

5 abzuschwächen, insbesondere die Expression zu verringern.

Weiterhin kann es für die Produktion von Aminosäuren vorteilhaft sein, neben der Verstärkung des *rpoB*-Gens unerwünschte Nebenreaktionen auszuschalten (Nakayama: "Breeding of Amino Acid Producing Micro-organisms", in: 10 Overproduction of Microbial Products, Krumphanzl, Sikyta, Vanek (eds.), Academic Press, London, UK, 1982).

Die erfindungsgemäß hergestellten Mikroorganismen sind ebenfalls Gegenstand der Erfindung und können kontinuierlich oder diskontinuierlich im batch - Verfahren 15 (Satzkultivierung) oder im fed batch (Zulaufverfahren) oder repeated fed batch Verfahren (repetitives Zulaufverfahren) zum Zwecke der Produktion von Aminosäuren kultiviert werden. Eine Zusammenfassung über bekannte Kultivierungsmethoden ist im Lehrbuch von Chmiel 20 (Bioprozeßtechnik 1. Einführung in die Bioverfahrenstechnik (Gustav Fischer Verlag, Stuttgart, 1991)) oder im Lehrbuch von Storhas (Bioreaktoren und periphere Einrichtungen (Vieweg Verlag, Braunschweig/Wiesbaden, 1994)) beschrieben.

Das zu verwendende Kulturmedium muß in geeigneter Weise den 25 Ansprüchen der jeweiligen Stämme genügen. Beschreibungen von Kulturmedien verschiedener Mikroorganismen sind im Handbuch "Manual of Methods for General Bacteriology" der American Society for Bacteriology (Washington D.C., USA, 1981) enthalten.

30 Als Kohlenstoffquelle können Zucker und Kohlehydrate wie z.B. Glucose, Saccharose, Lactose, Fructose, Maltose, Melasse, Stärke und Cellulose, Öle und Fette wie z.B. Sojaöl, Sonnenblumenöl, Erdnußöl und Kokosfett, Fettsäuren

wie z.B. Palmitinsäure, Stearinsäure und Linolsäure, Alkohole wie z.B. Glycerin und Ethanol und organische Säuren wie z.B. Essigsäure verwendet werden. Diese Stoffe können einzeln oder als Mischung verwendet werden.

- 5 Als Stickstoffquelle können organische Stickstoff-haltige Verbindungen wie Peptone, Hefeextrakt, Fleischextrakt, Malzextrakt, Maisquellwasser, Sojabohnenmehl und Harnstoff oder anorganische Verbindungen wie Ammoniumsulfat, Ammoniumchlorid, Ammoniumphosphat, Ammoniumcarbonat und
10 Ammoniumnitrat verwendet werden. Die Stickstoffquellen können einzeln oder als Mischung verwendet werden.

- Als Phosphorquelle können Phosphorsäure, Kaliumdihydrogenphosphat oder Dikaliumhydrogenphosphat oder die entsprechenden Natrium haltigen Salze verwendet werden. Das
15 Kulturmedium muß weiterhin Salze von Metallen enthalten wie z.B. Magnesiumsulfat oder Eisensulfat, die für das Wachstum notwendig sind. Schließlich können essentielle Wuchsstoffe wie Aminosäuren und Vitamine zusätzlich zu den oben genannten Stoffen eingesetzt werden. Dem Kulturmedium
20 können überdies geeignete Vorstufen zugesetzt werden. Die genannten Einsatzstoffe können zur Kultur in Form eines einmaligen Ansatzes hinzugegeben oder in geeigneter Weise während der Kultivierung zugefüttert werden.

- Zur pH-Kontrolle der Kultur werden basische Verbindungen
25 wie Natriumhydroxid, Kaliumhydroxid, Ammoniak bzw. Ammoniakwasser oder saure Verbindungen wie Phosphorsäure oder Schwefelsäure in geeigneter Weise eingesetzt. Zur Kontrolle der Schaumentwicklung können Antischaummittel wie z.B. Fettsäurepolyglykolester eingesetzt werden. Zur
30 Aufrechterhaltung der Stabilität von Plasmiden können dem Medium geeignete selektiv wirkende Stoffe wie z.B. Antibiotika hinzugefügt werden. Um aerobe Bedingungen aufrechtzuerhalten, werden Sauerstoff oder Sauerstoff haltige Gasmischungen wie z.B. Luft in die Kultur
35 eingetragen. Die Temperatur der Kultur liegt normalerweise

bei 20°C bis 45°C und vorzugsweise bei 25°C bis 40°C. Die Kultur wird solange fortgesetzt, bis sich ein Maximum des gewünschten Produktes gebildet hat. Dieses Ziel wird normalerweise innerhalb von 10 Stunden bis 160 Stunden erreicht.

Methoden zur Bestimmung von L-Aminosäuren sind aus dem Stand der Technik bekannt. Die Analyse kann zum Beispiel so wie bei Spackman et al. (Analytical Chemistry, 30, (1958), 1190) beschrieben durch Ionenaustausch-Chromatographie mit anschließender Ninhydrin-Derivatisierung erfolgen, oder sie kann durch reversed phase HPLC erfolgen, so wie bei Lindroth et al. (Analytical Chemistry (1979) 51: 1167-1174) beschrieben.

Reinkulturen folgender Mikroorganismen wurden bei der Deutschen Sammlung für Mikroorganismen und Zellkulturen (DSMZ, Braunschweig, Deutschland) am 16. Januar 2001 gemäß Budapester Vertrag hinterlegt:

- *Corynebacterium glutamicum* Stamm DM1546 als DSM 13993
- *Corynebacterium glutamicum* Stamm DM1547 als DSM 13994

Das erfindungsgemäße Verfahren dient zur fermentativen Herstellung von Aminosäuren.

Die vorliegende Erfindung wird im folgenden anhand von Ausführungsbeispielen näher erläutert.

Die Isolierung von Plasmid-DNA aus *Escherichia coli* sowie alle Techniken zur Restriktion, Klenow- und alkalische Phosphatasebehandlung wurden nach Sambrook et al. (Molecular Cloning. A Laboratory Manual (1989) Cold Spring Harbour Laboratory Press, Cold Spring Harbor, NY, USA) durchgeführt. Methoden zur Transformation von *Escherichia coli* sind ebenfalls in diesem Handbuch beschrieben.

Die Zusammensetzung gängiger Nährmedien wie LB- oder TY-Medium kann ebenfalls dem Handbuch von Sambrook et al. entnommen werden.

Beispiel 1

- 5 Herstellung einer genomischen Cosmid-Genbank aus
Corynebacterium glutamicum ATCC 13032

Chromosomale DNA aus Corynebacterium glutamicum ATCC 13032 wird wie bei Tauch et al. (1995, Plasmid 33:168-179) beschrieben isoliert und mit dem Restriktionsenzym Sau3AI
10 (Amersham Pharmacia, Freiburg, Deutschland, Produktbeschreibung Sau3AI, Code no. 27-0913-02) partiell gespalten. Die DNA-Fragmente werden mit shrimp alkalischer Phosphatase (Roche Diagnostics GmbH, Mannheim, Deutschland, Produktbeschreibung SAP, Code no. 1758250)
15 dephosphoryliert. Die DNA des Cosmid-Vektors SuperCos1 (Wahl et al. (1987) Proceedings of the National Academy of Sciences USA 84:2160-2164), bezogen von der Firma Stratagene (La Jolla, USA, Produktbeschreibung SuperCos1 Cosmid Vektor Kit, Code no. 251301) wird mit dem
20 Restriktionsenzym XbaI (Amersham Pharmacia, Freiburg, Deutschland, Produktbeschreibung XbaI, Code no. 27-0948-02) gespalten und ebenfalls mit shrimp alkalischer Phosphatase dephosphoryliert.

Anschließend wird die Cosmid-DNA mit dem Restriktionsenzym
25 BamHI (Amersham Pharmacia, Freiburg, Deutschland, Produktbeschreibung BamHI, Code no. 27-0868-04) gespalten. Die auf diese Weise behandelte Cosmid-DNA wird mit der behandelten ATCC13032-DNA gemischt und der Ansatz mit T4-DNA-Ligase (Amersham Pharmacia, Freiburg, Deutschland,
30 Produktbeschreibung T4-DNA-Ligase, Code no. 27-0870-04) behandelt. Das Ligationsgemisch wird anschließend mit Hilfe des Gigapack II XL Packing Extracts (Stratagene, La Jolla, USA, Produktbeschreibung Gigapack II XL Packing Extract, Code no. 200217) in Phagen verpackt.

Zur Infektion des E. coli Stammes NM554 (Raleigh et al. 1988, Nucleic Acid Research 16:1563-1575) werden die Zellen in 10 mM MgSO₄ aufgenommen und mit einem Aliquot der Phagensuspension vermischt. Infektion und Titerung der Cosmidbank werden wie bei Sambrook et al. (1989, Molecular Cloning: A Laboratory Manual, Cold Spring Harbor) beschrieben durchgeführt, wobei die Zellen auf LB-Agar (Lennox, 1955, Virology, 1:190) mit 100 mg/l Ampicillin ausplattiert werden. Nach Inkubation über Nacht bei 37°C werden rekombinante Einzelklone selektioniert.

Beispiel 2

Isolierung und Sequenzierung des rpoB-Gens

Die Cosmid-DNA einer Einzelkolonie wird mit dem Qiaprep Spin Miniprep Kit (Product No. 27106, Qiagen, Hilden, Germany) nach Herstellerangaben isoliert und mit dem Restriktionsenzym Sau3AI (Amersham Pharmacia, Freiburg, Deutschland, Produktbeschreibung Sau3AI, Product No. 27-0913-02) partiell gespalten. Die DNA-Fragmente werden mit shrimp alkalischer Phosphatase (Roche Diagnostics GmbH, Mannheim, Deutschland, Produktbeschreibung SAP, Product No. 1758250) dephosphoryliert. Nach gelelektrophoretischer Auftrennung erfolgt die Isolierung der Cosmidfragmente im Größenbereich von 1500 bis 2000 bp mit dem QiaExII Gel Extraction Kit (Product No. 20021, Qiagen, Hilden, Germany).

Die DNA des Sequenziervektors pZero-1, bezogen von der Firma Invitrogen (Groningen, Niederlande, Produktbeschreibung Zero Background Cloning Kit, Product No. K2500-01), wird mit dem Restriktionsenzym BamHI (Amersham Pharmacia, Freiburg, Deutschland, Produktbeschreibung BamHI, Product No. 27-0868-04) gespalten. Die Ligation der Cosmidfragmente in den Sequenziervektor pZero-1 wird wie von Sambrook et al. (1989, Molecular Cloning: A Laboratory Manual, Cold Spring

Harbor) beschrieben durchgeführt, wobei das DNA-Gemisch mit T4-Ligase (Pharmacia Biotech, Freiburg, Deutschland) über Nacht inkubiert wird. Dieses Ligationsgemisch wird anschließend in den E. coli Stamm DH5 α MCR (Grant, 1990, 5 Proceedings of the National Academy of Sciences U.S.A., 87:4645-4649) elektroporiert (Tauch et al. 1994, FEMS Microbiol Letters, 123:343-7) und auf LB-Agar (Lennox, 1955, Virology, 1:190) mit 50 mg/l Zeocin ausplattiert.

Die Plasmidpräparation der rekombinanten Klone erfolgt mit 10 dem Biorobot 9600 (Product No. 900200, Qiagen, Hilden, Deutschland). Die Sequenzierung erfolgt nach der Dideoxy-Kettenabbruch-Methode von Sanger et al. (1977, Proceedings of the National Academy of Sciences U.S.A., 74:5463-5467) mit Modifikationen nach Zimmermann et al. (1990, Nucleic 15 Acids Research, 18:1067). Es wird der "RR dRhodamin Terminator Cycle Sequencing Kit" von PE Applied Biosystems (Product No. 403044, Weiterstadt, Deutschland) verwendet. Die gelelektrophoretische Auftrennung und Analyse der Sequenzierreaktion erfolgt in einem "Rotiphorese NF 20 Acrylamid/Bisacrylamid" Gel (29:1) (Product No. A124.1, Roth, Karlsruhe, Germany) mit dem "ABI Prism 377" Sequenziergerät von PE Applied Biosystems (Weiterstadt, Deutschland).

Die erhaltenen Roh-Sequenzdaten werden anschließend unter 25 Anwendung des Staden-Programmpakets (1986, Nucleic Acids Research, 14:217-231) Version 97-0 prozessiert. Die Einzelsequenzen der pZerol-Derivate werden zu einem zusammenhängenden Contig assembliert. Die computergestützte Kodierbereichsanalyse wird mit dem Programm XNIP (Staden, 30 1986, Nucleic Acids Research, 14:217-231) angefertigt.

Die erhaltene Nukleotidsequenz ist in SEQ ID No. 1 dargestellt. Die Analyse der Nukleotidsequenz ergibt ein offenes Leseraster von 3497 Basenpaaren, welches als rpoB-Gen bezeichnet wird. Das rpoB-Gen kodiert für ein Protein 35 von 1165 Aminosäuren.

SEQUENZPROTOKOLL

<110> Degussa-Hüls AG

5 <120> Neue für das rpoB-Gen kodierende Nukleotidsequenzen

<130> 000781 BT

<140>

10 <141>

<160> 8

<170> PatentIn Ver. 2.1

15

<210> 1

<211> 5099

<212> DNA

<213> Corynebacterium glutamicum

20

<220>

<221> CDS

<222> (702)..(4196)

<223> rpoB-Wildtypgen

25

<400> 1

acaatgtgac tcgtgatttt tgggtggatc agcgtaccgg tttggttgtc gatctagctg 60

30

aaaatattga tgattttttac ggcgaccgca gcggccagaa gtacgaacag aaattgcttt 120

tcgacgcctc cctcgacgat gcagctgtct ctaagctggg tgcacaggcc gaaagcatcc 180

ctgatggaga tgtgagcaaa atcgcaaata ccgtaggtat tgtgatcggt gcggtattgg 240

35

ctctcgtggg cctggccggg tgttttgggg cgtttgggaa gaaacgtcga gaagcttaac 300

ctgctgttca aatagatttt ccctgtttcg aattgcggaa accccgggtt tgtttgctag 360

40

ggtgcctcgt agaaggggtc aagaagattt ctgggaaacg cgcccgtgcg gttgggttgc 420

aatagcacgc ggagcaccag atgaaaaatc tcccctttac tttcgcgcg c gattgggtata 480

ctctgagtcg ttgcgttgga attcgtgact ctttttcgtt cctgtagcgc caagaccttg 540

45

atcaaggtgg tttaaaaaaa ccgatttgac aaggtcattc agtgctatct ggagtcgttc 600

aggggggatcg gggttcctcag cagaccaatt gctcaaaaat accagcgggtg ttgatctgca 660

50

cttaatggcc ttgaccagcc aggtgcaatt acccgcgtga g gtg ctg gaa gga ccc 716

Met Leu Glu Gly Pro

1

5

atc ttg gca gtc tcc cgc cag acc aag tca gtc gtc gat att ccc ggt 764

Ile Leu Ala Val Ser Arg Gln Thr Lys Ser Val Val Asp Ile Pro Gly

55

10

15

20

gca ccg cag cgt tat tct ttc gcg aag gtg tcc gca ccc att gag gtg 812

Ala Pro Gln Arg Tyr Ser Phe Ala Lys Val Ser Ala Pro Ile Glu Val

25

30

35

	ccc ggg cta cta gat ctt caa ctg gat tct tac tcc tgg ctg att ggt	860
	Pro Gly Leu Leu Asp Leu Gln Leu Asp Ser Tyr Ser Trp Leu Ile Gly	
	40 45 50	
5	acg cct gag tgg cgt gct cgt cag aag gaa gaa ttc ggc gag gga gcc	908
	Thr Pro Glu Trp Arg Ala Arg Gln Lys Glu Glu Phe Gly Glu Gly Ala	
	55 60 65	
10	cgc gta acc agc ggc ctt gag aac att ctc gag gag ctc tcc cca atc	956
	Arg Val Thr Ser Gly Leu Glu Asn Ile Leu Glu Glu Leu Ser Pro Ile	
	70 75 80 85	
15	cag gat tac tct gga aac atg tcc ctg agc ctt tcg gag cca cgc ttc	1004
	Gln Asp Tyr Ser Gly Asn Met Ser Leu Ser Leu Ser Glu Pro Arg Phe	
	90 95 100	
20	gaa gac gtc aag aac acc att gac gag gcg aaa gaa aag gac atc aac	1052
	Glu Asp Val Lys Asn Thr Ile Asp Glu Ala Lys Glu Lys Asp Ile Asn	
	105 110 115	
25	tac gcg gcg cca ctg tat gtg acc gcg gag ttc gtc aac aac acc acc	1100
	Tyr Ala Ala Pro Leu Tyr Val Thr Ala Glu Phe Val Asn Asn Thr Thr	
	120 125 130	
30	ggt gaa atc aag tct cag act gtc ttc atc ggc gat ttc cca atg atg	1148
	Gly Glu Ile Lys Ser Gln Thr Val Phe Ile Gly Asp Phe Pro Met Met	
	135 140 145	
35	acg gac aag gga acg ttc atc atc aac gga acc gaa cgc gtt gtg gtc	1196
	Thr Asp Lys Gly Thr Phe Ile Ile Asn Gly Thr Glu Arg Val Val Val	
	150 155 160 165	
40	agc cag ctc gtc cgc tcc ccg ggc gtg tac ttt gac cag acc atc gat	1244
	Ser Gln Leu Val Arg Ser Pro Gly Val Tyr Phe Asp Gln Thr Ile Asp	
	170 175 180	
45	aag tca act gag cgt cca ctg cac gcc gtg aag gtt att cct tcc cgt	1292
	Lys Ser Thr Glu Arg Pro Leu His Ala Val Lys Val Ile Pro Ser Arg	
	185 190 195	
50	ggt gct tgg ctt gag ttt gac gtc gat aag cgc gat tcg gtt ggt gtt	1340
	Gly Ala Trp Leu Glu Phe Asp Val Asp Lys Arg Asp Ser Val Gly Val	
	200 205 210	
55	cgt att gac cgc aag cgt cgc cag cca gtc acc gta ctg ctg aag gct	1388
	Arg Ile Asp Arg Lys Arg Arg Gln Pro Val Thr Val Leu Leu Lys Ala	
	215 220 225	
60	ctt ggc tgg acc act gag cag atc acc gag cgt ttc ggt ttc tct gaa	1436
	Leu Gly Trp Thr Thr Glu Gln Ile Thr Glu Arg Phe Gly Phe Ser Glu	
	230 235 240 245	
65	atc atg atg tcc acc ctc gag tcc gat ggt gta gca aac acc gat gag	1484
	Ile Met Met Ser Thr Leu Glu Ser Asp Gly Val Ala Asn Thr Asp Glu	
	250 255 260	

		gca	ttg	ctg	gag	atc	tac	cgc	aag	cag	cgt	cca	ggc	gag	cag	cct	acc	1532
		Ala	Leu	Leu	Glu	Ile	Tyr	Arg	Lys	Gln	Arg	Pro	Gly	Glu	Gln	Pro	Thr	
					265					270						275		
5		cgc	gac	ctt	gcg	cag	tcc	ctc	ctg	gac	aac	agc	ttc	ttc	cgt	gca	aag	1580
		Arg	Asp	Leu	Ala	Gln	Ser	Leu	Leu	Asp	Asn	Ser	Phe	Phe	Arg	Ala	Lys	
				280					285						290			
10		cgc	tac	gac	ctg	gct	cgc	ggt	ggt	cgt	tac	aag	atc	aac	cgc	aag	ctc	1628
		Arg	Tyr	Asp	Leu	Ala	Arg	Val	Gly	Arg	Tyr	Lys	Ile	Asn	Arg	Lys	Leu	
			295					300					305					
15		ggc	ctt	ggt	ggc	gac	cac	gat	ggt	ttg	atg	act	ctt	act	gaa	gag	gac	1676
		Gly	Leu	Gly	Gly	Asp	His	Asp	Gly	Leu	Met	Thr	Leu	Thr	Glu	Glu	Asp	
		310					315					320					325	
		atc	gca	acc	acc	atc	gag	tac	ctg	gtg	cgt	ctg	cac	gca	ggt	gag	cgc	1724
		Ile	Ala	Thr	Thr	Ile	Glu	Tyr	Leu	Val	Arg	Leu	His	Ala	Gly	Glu	Arg	
						330					335					340		
20		gtc	atg	act	tct	cca	aat	ggt	gaa	gag	atc	cca	gtc	gag	acc	gat	gac	1772
		Val	Met	Thr	Ser	Pro	Asn	Gly	Glu	Glu	Ile	Pro	Val	Glu	Thr	Asp	Asp	
					345				350						355			
25		atc	gac	cac	ttt	ggt	aac	cgt	cgt	ctg	cgt	acc	ggt	ggc	gaa	ctg	atc	1820
		Ile	Asp	His	Phe	Gly	Asn	Arg	Arg	Leu	Arg	Thr	Val	Gly	Glu	Leu	Ile	
				360				365						370				
30		cag	aac	cag	gtc	cgt	gtc	ggc	ctg	tcc	cgc	atg	gag	cgc	ggt	ggt	cgt	1868
		Gln	Asn	Gln	Val	Arg	Val	Gly	Leu	Ser	Arg	Met	Glu	Arg	Val	Val	Arg	
			375					380					385					
35		gag	cgt	atg	acc	acc	cag	gat	gcg	gag	tcc	att	act	cct	act	tcc	ttg	1916
		Glu	Arg	Met	Thr	Thr	Gln	Asp	Ala	Glu	Ser	Ile	Thr	Pro	Thr	Ser	Leu	
		390					395					400					405	
		atc	aac	ggt	cgt	cct	gtc	tct	gca	gct	atc	cgt	gag	ttc	ttc	gga	act	1964
		Ile	Asn	Val	Arg	Pro	Val	Ser	Ala	Ala	Ile	Arg	Glu	Phe	Phe	Gly	Thr	
						410					415					420		
40		tcc	cag	ctg	tct	cag	ttc	atg	gac	cag	aac	aac	tcc	ctg	tct	ggt	ttg	2012
		Ser	Gln	Leu	Ser	Gln	Phe	Met	Asp	Gln	Asn	Asn	Ser	Leu	Ser	Gly	Leu	
					425				430						435			
45		act	cac	aag	cgt	cgt	ctg	tcg	gct	ctg	ggc	ccg	ggt	ggt	ctg	tcc	cgt	2060
		Thr	His	Lys	Arg	Arg	Leu	Ser	Ala	Leu	Gly	Pro	Gly	Gly	Leu	Ser	Arg	
				440					445					450				
50		gag	cgc	gcc	ggc	atc	gag	gtt	cga	gac	gtt	cac	cca	tct	cac	tac	ggc	2108
		Glu	Arg	Ala	Gly	Ile	Glu	Val	Arg	Asp	Val	His	Pro	Ser	His	Tyr	Gly	
			455					460					465					
55		cgt	atg	tgc	cca	att	gag	act	ccg	gaa	ggt	cca	aac	att	ggc	ctg	atc	2156
		Arg	Met	Cys	Pro	Ile	Glu	Thr	Pro	Glu	Gly	Pro	Asn	Ile	Gly	Leu	Ile	
		470					475					480					485	
		ggt	tcc	ttg	gct	tcc	tat	gct	cga	gtg	aac	cca	ttc	ggt	ttc	att	gag	2204
		Gly	Ser	Leu	Ala	Ser	Tyr	Ala	Arg	Val	Asn	Pro	Phe	Gly	Phe	Ile	Glu	
						490					495					500		

5	acc cca tac cgt cgc atc atc gac ggc aag ctg acc gac cag att gac Thr Pro Tyr Arg Arg Ile Ile Asp Gly Lys Leu Thr Asp Gln Ile Asp 505 510 515	2252
10	tac ctt acc gct gat gag gaa gac cgc ttc gtt gtt gcg cag gca aac Tyr Leu Thr Ala Asp Glu Glu Asp Arg Phe Val Val Ala Gln Ala Asn 520 525 530	2300
15	acg cac tac gac gaa gag ggc aac atc acc gat gag acc gtc act gtt Thr His Tyr Asp Glu Glu Gly Asn Ile Thr Asp Glu Thr Val Thr Val 535 540 545	2348
20	cgt ctg aag gac ggc gac atc gcc atg gtt ggc cgc aac gcg gtt gat Arg Leu Lys Asp Gly Asp Ile Ala Met Val Gly Arg Asn Ala Val Asp 550 555 560 565	2396
25	tac atg gac gtt tcc cct cgt cag atg gtt tct gtt ggt acc gcg atg Tyr Met Asp Val Ser Pro Arg Gln Met Val Ser Val Gly Thr Ala Met 570 575 580	2444
30	att cca ttc ctg gag cac gac gat gct aac cgt gca ctg atg ggc gcg Ile Pro Phe Leu Glu His Asp Asp Ala Asn Arg Ala Leu Met Gly Ala 585 590 595	2492
35	aac atg cag aag cag gct gtg cca ctg att cgt gcc gag gct cct ttc Asn Met Gln Lys Gln Ala Val Pro Leu Ile Arg Ala Glu Ala Pro Phe 600 605 610	2540
40	gtg ggc acc ggt atg gag cag cgc gca gca tac gac gcc ggc gac ctg Val Gly Thr Gly Met Glu Gln Arg Ala Ala Tyr Asp Ala Gly Asp Leu 615 620 625	2588
45	gtt att acc cca gtc gca ggt gtg gtg gaa aac gtt tca gct gac ttc Val Ile Thr Pro Val Ala Gly Val Val Glu Asn Val Ser Ala Asp Phe 630 635 640 645	2636
50	atc acc atc atg gct gat gac ggc aag cgc gaa acc tac ctg ctg cgt Ile Thr Ile Met Ala Asp Asp Gly Lys Arg Glu Thr Tyr Leu Leu Arg 650 655 660	2684
55	aag ttc cag cgc acc aac cag ggc acc agc tac aac cag aag cct ttg Lys Phe Gln Arg Thr Asn Gln Gly Thr Ser Tyr Asn Gln Lys Pro Leu 665 670 675	2732
60	gtt aac ttg ggc gag cgc gtt gaa gct ggc cag gtt att gct gat ggt Val Asn Leu Gly Glu Arg Val Glu Ala Gly Gln Val Ile Ala Asp Gly 680 685 690	2780
65	cca ggt acc ttc aat ggt gaa atg tcc ctt ggc cgt aac ctt ctg gtt Pro Gly Thr Phe Asn Gly Glu Met Ser Leu Gly Arg Asn Leu Leu Val 695 700 705	2828
70	gcg ttc atg cct tgg gaa ggc cac aac tac gag gat gcg atc atc ctc Ala Phe Met Pro Trp Glu Gly His Asn Tyr Glu Asp Ala Ile Ile Leu 710 715 720 725	2876

	aac cag aac atc gtt gag cag gac atc ttg acc tcg atc cac atc gag	2924
	Asn Gln Asn Ile Val Glu Gln Asp Ile Leu Thr Ser Ile His Ile Glu	
	730 735 740	
5	gag cac gag atc gat gcc cgc gac act aag ctt ggc gcc gaa gaa atc	2972
	Glu His Glu Ile Asp Ala Arg Asp Thr Lys Leu Gly Ala Glu Glu Ile	
	745 750 755	
10	acc cgc gac atc cct aat gtg tct gaa gaa gtc ctc aag gac ctc gac	3020
	Thr Arg Asp Ile Pro Asn Val Ser Glu Glu Val Leu Lys Asp Leu Asp	
	760 765 770	
15	gac cgc ggt att gtc cgc atc ggt gct gat gtt cgt gac ggc gac atc	3068
	Asp Arg Gly Ile Val Arg Ile Gly Ala Asp Val Arg Asp Gly Asp Ile	
	775 780 785	
20	ctg gtc ggt aag gtc acc cct aag ggc gag acc gag ctc acc ccg gaa	3116
	Leu Val Gly Lys Val Thr Pro Lys Gly Glu Thr Glu Leu Thr Pro Glu	
	790 795 800 805	
25	gag cgc ttg ctg cgc gca atc ttc ggt gag aag gcc cgc gaa gtt cgc	3164
	Glu Arg Leu Leu Arg Ala Ile Phe Gly Glu Lys Ala Arg Glu Val Arg	
	810 815 820	
30	gat acc tcc atg aag gtg cct cac ggt gag acc ggc aag gtc atc ggc	3212
	Asp Thr Ser Met Lys Val Pro His Gly Glu Thr Gly Lys Val Ile Gly	
	825 830 835	
35	gtg cgt cac ttc tcc cgc gag gac gac gac gat ctg gct cct ggc gtc	3260
	Val Arg His Phe Ser Arg Glu Asp Asp Asp Asp Leu Ala Pro Gly Val	
	840 845 850	
40	aac gag atg atc cgt atc tac gtt gct cag aag cgt aag atc cag gac	3308
	Asn Glu Met Ile Arg Ile Tyr Val Ala Gln Lys Arg Lys Ile Gln Asp	
	855 860 865	
45	ggc gat aag ctc gct ggc cgc cac ggt aac aag ggt gtt gtc ggt aaa	3356
	Gly Asp Lys Leu Ala Gly Arg His Gly Asn Lys Gly Val Val Gly Lys	
	870 875 880 885	
50	att ttg cct cag gaa gat atg cca ttc ctt cca gac ggc act cct gtt	3404
	Ile Leu Pro Gln Glu Asp Met Pro Phe Leu Pro Asp Gly Thr Pro Val	
	890 895 900	
55	gac atc atc ttg aac acc cac ggt gtt cca cgt cgt atg aac att ggt	3452
	Asp Ile Ile Leu Asn Thr His Gly Val Pro Arg Arg Met Asn Ile Gly	
	905 910 915	
60	cag gtt ctt gag acc cac ctt ggc tgg ctg gca tct gct ggt tgg tcc	3500
	Gln Val Leu Glu Thr His Leu Gly Trp Leu Ala Ser Ala Gly Trp Ser	
	920 925 930	
65	gtg gat cct gaa gat cct gag aac gct gag ctc gtc aag act ctg cct	3548
	Val Asp Pro Glu Asp Pro Glu Asn Ala Glu Leu Val Lys Thr Leu Pro	
	935 940 945	
70	gca gac ctc ctc gag gtt cct gct ggt tcc ttg act gca act cct gtg	3596
	Ala Asp Leu Leu Glu Val Pro Ala Gly Ser Leu Thr Ala Thr Pro Val	
	950 955 960 965	

ttc gac ggt gcg tca aac gaa gag ctc gca ggc ctg ctc gct aat tca 3644
 Phe Asp Gly Ala Ser Asn Glu Glu Leu Ala Gly Leu Leu Ala Asn Ser
 970 975 980

5

cgt cca aac cgc gac ggc gac gtc atg gtt aac gcg gat ggt aaa gca 3692
 Arg Pro Asn Arg Asp Gly Asp Val Met Val Asn Ala Asp Gly Lys Ala
 985 990 995

10

acg ctt atc gac ggt cgc tcc ggt gag cct tac ccg tac ccg gtt tcc 3740
 Thr Leu Ile Asp Gly Arg Ser Gly Glu Pro Tyr Pro Tyr Pro Val Ser
 1000 1005 1010

15

atc ggc tac atg tac atg ctg aag ctg cac cac ctc gtt gac gag aag 3788
 Ile Gly Tyr Met Tyr Met Leu Lys Leu His His Leu Val Asp Glu Lys
 1015 1020 1025

20

atc cac gca cgt tcc act ggt cct tac tcc atg att acc cag cag cca 3836
 Ile His Ala Arg Ser Thr Gly Pro Tyr Ser Met Ile Thr Gln Gln Pro
 1030 1035 1040 1045

ctg ggt ggt aaa gca cag ttc ggt gga cag cgt ttc ggc gaa atg gag 3884
 Leu Gly Gly Lys Ala Gln Phe Gly Gly Gln Arg Phe Gly Glu Met Glu
 1050 1055 1060

gtg tgg gca atg cag gca tac ggc gct gcc tac aca ctt cag gag ctg 3932
 Val Trp Ala Met Gln Ala Tyr Gly Ala Ala Tyr Thr Leu Gln Glu Leu
 1065 1070 1075

30

ctg acc atc aag tct gat gac gtg gtt ggc cgt gtc aag gtc tac gaa 3980
 Leu Thr Ile Lys Ser Asp Asp Val Val Gly Arg Val Lys Val Tyr Glu
 1080 1085 1090

35

gca att gtg aag ggc gag aac atc ccg gat cca ggt att cct gag tcc 4028
 Ala Ile Val Lys Gly Glu Asn Ile Pro Asp Pro Gly Ile Pro Glu Ser
 1095 1100 1105

40

ttc aag gtt ctc ctc aag gag ctc cag tcc ttg tgc ctg aac gtg gag 4076
 Phe Lys Val Leu Leu Lys Glu Leu Gln Ser Leu Cys Leu Asn Val Glu
 1110 1115 1120 1125

45

gtt ctc tcc gca gac ggc act cca atg gag ctc gcg ggt gac gac gac 4124
 Val Leu Ser Ala Asp Gly Thr Pro Met Glu Leu Ala Gly Asp Asp Asp
 1130 1135 1140

gac ttc gat cag gca ggc gcc tca ctt ggc atc aac ctg tcc cgt gac 4172
 Asp Phe Asp Gln Ala Gly Ala Ser Leu Gly Ile Asn Leu Ser Arg Asp
 1145 1150 1155

50

gag cgt tcc gac gcc gac acc gca tagcagatca gaaaacaacc gctagaaatc 4226
 Glu Arg Ser Asp Ala Asp Thr Ala
 1160 1165

55

aagccataca tcccccgac attgaagaga tggtctgggg ggaaaggag ttttacgtgc 4286
 tcgacgtaaa cgtcttcgat gagctccgca tcggcctggc caccgccgac gacatccgcc 4346
 gttggtccaa ggggtgaggtc aagaagccgg agaccatcaa ctaccgaacc ctcaagcctg 4406

agaaggacgg tctgttctgc gagcgtatct tcggtccaac tcgcgactgg gagtgcgcct 4466
 gcggtaagta caagcgtgtc cgctacaagg gcatcatctg tgaacgctgt ggcgttgagg 4526
 5 tcaccaagtc caaggtgcgc cgtgagcgca tgggacacat tgagctcgct gcaccagtaa 4586
 cccacatttg gtacttcaag ggcgttccat cacgcctcgg ctaccttttg gaccttgctc 4646
 10 caaaggacct ggacctcatc atctacttcg gtgcgaacat catcaccagc gtggacgaag 4706
 aggctcgcca cagcgaccag accactcttg aggagaaat gcttctggag aagaaggacg 4766
 ttgaggcaga cgcagagtct gacattgctg agcgtgctga aaagctcgaa gaggatcttg 4826
 15 ctgaacttga ggcagctggc gctaaggccg acgctcgccg caaggttcag gctgctgccg 4886
 ataaggaaat gcagcacatc cgtgagcgtg cacagcgcca aatcgatcgt ctcgatgagg 4946
 20 tctggcagac cttcatcaag cttgctccaa agcagatgat ccgcatgag aagctctacg 5006
 atgaactgat cgaccgctac gaggattact tcaccggtgg tatgggtgca gagtccattg 5066
 aggctttgat ccagaacttc gaccttgatg ctg 5099

25

<210> 2

<211> 1165

<212> PRT

<213> Corynebacterium glutamicum

30

<400> 2

Met Leu Glu Gly Pro Ile Leu Ala Val Ser Arg Gln Thr Lys Ser Val
 1 5 10 15

35

Val Asp Ile Pro Gly Ala Pro Gln Arg Tyr Ser Phe Ala Lys Val Ser
 20 25 30

Ala Pro Ile Glu Val Pro Gly Leu Leu Asp Leu Gln Leu Asp Ser Tyr
 35 40 45

40

Ser Trp Leu Ile Gly Thr Pro Glu Trp Arg Ala Arg Gln Lys Glu Glu
 50 55 60

45

Phe Gly Glu Gly Ala Arg Val Thr Ser Gly Leu Glu Asn Ile Leu Glu
 65 70 75 80

Glu Leu Ser Pro Ile Gln Asp Tyr Ser Gly Asn Met Ser Leu Ser Leu
 85 90 95

50

Ser Glu Pro Arg Phe Glu Asp Val Lys Asn Thr Ile Asp Glu Ala Lys
 100 105 110

Glu Lys Asp Ile Asn Tyr Ala Ala Pro Leu Tyr Val Thr Ala Glu Phe
 115 120 125

55

Val Asn Asn Thr Thr Gly Glu Ile Lys Ser Gln Thr Val Phe Ile Gly
 130 135 140

	Asp	Phe	Pro	Met	Met	Thr	Asp	Lys	Gly	Thr	Phe	Ile	Ile	Asn	Gly	Thr	145	150	155	160
5	Glu	Arg	Val	Val	Val	Ser	Gln	Leu	Val	Arg	Ser	Pro	Gly	Val	Tyr	Phe	165	170		175
	Asp	Gln	Thr	Ile	Asp	Lys	Ser	Thr	Glu	Arg	Pro	Leu	His	Ala	Val	Lys	180	185		190
10	Val	Ile	Pro	<u>Ser</u>	Arg	Gly	Ala	Trp	Leu	Glu	Phe	Asp	Val	Asp	Lys	Arg	195	200		205
	Asp	Ser	Val	Gly	Val	Arg	Ile	Asp	Arg	Lys	Arg	Arg	Gln	Pro	Val	Thr	210	215		220
15	Val	Leu	Leu	Lys	Ala	Leu	Gly	Trp	Thr	Thr	Glu	Gln	Ile	Thr	Glu	Arg	225	230	235	240
	Phe	Gly	Phe	Ser	Glu	Ile	Met	Met	Ser	Thr	Leu	Glu	Ser	Asp	Gly	Val	245	250		255
20	Ala	Asn	Thr	Asp	Glu	Ala	Leu	Leu	Glu	Ile	Tyr	Arg	Lys	Gln	Arg	Pro	260	265		270
25	Gly	Glu	Gln	Pro	Thr	Arg	Asp	Leu	Ala	Gln	Ser	Leu	Leu	Asp	Asn	Ser	275	280		285
	Phe	Phe	Arg	Ala	Lys	Arg	Tyr	Asp	Leu	Ala	Arg	Val	Gly	Arg	Tyr	Lys	290	295	300	
30	Ile	Asn	Arg	Lys	Leu	Gly	Leu	Gly	Gly	Asp	His	Asp	Gly	Leu	Met	Thr	305	310	315	320
	Leu	Thr	Glu	Glu	Asp	Ile	Ala	Thr	Thr	Ile	Glu	Tyr	Leu	Val	Arg	Leu	325	330		335
35	His	Ala	Gly	Glu	Arg	Val	Met	Thr	Ser	Pro	Asn	Gly	Glu	Glu	Ile	Pro	340	345		350
40	Val	Glu	Thr	Asp	Asp	Ile	Asp	His	Phe	Gly	Asn	Arg	Arg	Leu	Arg	Thr	355	360		365
	Val	Gly	Glu	Leu	Ile	Gln	Asn	Gln	Val	Arg	Val	Gly	Leu	Ser	Arg	Met	370	375	380	
45	Glu	Arg	Val	Val	Arg	Glu	Arg	Met	Thr	Thr	Gln	Asp	Ala	Glu	Ser	Ile	385	390	395	400
	Thr	Pro	Thr	Ser	Leu	Ile	Asn	Val	Arg	Pro	Val	Ser	Ala	Ala	Ile	Arg	405	410		415
50	Glu	Phe	Phe	Gly	Thr	Ser	Gln	Leu	Ser	Gln	Phe	Met	<u>Asp</u>	Gln	Asn	Asn	420	425		430
55	Ser	Leu	Ser	Gly	Leu	Thr	<u>His</u>	Lys	Arg	Arg	Leu	Ser	Ala	Leu	Gly	Pro	435	440		445
	Gly	Gly	Leu	Ser	Arg	Glu	Arg	Ala	Gly	Ile	Glu	Val	Arg	Asp	Val	His	450	455	460	

	Pro	Ser	His	Tyr	Gly	Arg	Met	Cys	Pro	Ile	Glu	Thr	Pro	Glu	Gly	Pro	465	470	475	480
5	Asn	Ile	Gly	Leu	Ile	Gly	Ser	Leu	Ala	Ser	Tyr	Ala	Arg	Val	Asn	Pro		485	490	495
	Phe	Gly	Phe	Ile	Glu	Thr	Pro	Tyr	Arg	Arg	Ile	Ile	Asp	Gly	Lys	Leu		500	505	510
10	Thr	Asp	Gln	Ile	Asp	Tyr	Leu	Thr	Ala	Asp	Glu	Glu	Asp	Arg	Phe	Val		515	520	525
15	Val	Ala	Gln	Ala	Asn	Thr	His	Tyr	Asp	Glu	Glu	Gly	Asn	Ile	Thr	Asp		530	535	540
	Glu	Thr	Val	Thr	Val	Arg	Leu	Lys	Asp	Gly	Asp	Ile	Ala	Met	Val	Gly		545	550	555
20	Arg	Asn	Ala	Val	Asp	Tyr	Met	Asp	Val	Ser	Pro	Arg	Gln	Met	Val	Ser		565	570	575
	Val	Gly	Thr	Ala	Met	Ile	Pro	Phe	Leu	Glu	His	Asp	Asp	Ala	Asn	Arg		580	585	590
25	Ala	Leu	Met	Gly	Ala	Asn	Met	Gln	Lys	Gln	Ala	Val	Pro	Leu	Ile	Arg		595	600	605
30	Ala	Glu	Ala	Pro	Phe	Val	Gly	Thr	Gly	Met	Glu	Gln	Arg	Ala	Ala	Tyr		610	615	620
	Asp	Ala	Gly	Asp	Leu	Val	Ile	Thr	Pro	Val	Ala	Gly	Val	Val	Glu	Asn		625	630	635
35	Val	Ser	Ala	Asp	Phe	Ile	Thr	Ile	Met	Ala	Asp	Asp	Gly	Lys	Arg	Glu		645	650	655
	Thr	Tyr	Leu	Leu	Arg	Lys	Phe	Gln	Arg	Thr	Asn	Gln	Gly	Thr	Ser	Tyr		660	665	670
40	Asn	Gln	Lys	Pro	Leu	Val	Asn	Leu	Gly	Glu	Arg	Val	Glu	Ala	Gly	Gln		675	680	685
45	Val	Ile	Ala	Asp	Gly	Pro	Gly	Thr	Phe	Asn	Gly	Glu	Met	Ser	Leu	Gly		690	695	700
	Arg	Asn	Leu	Leu	Val	Ala	Phe	Met	Pro	Trp	Glu	Gly	His	Asn	Tyr	Glu		705	710	715
50	Asp	Ala	Ile	Ile	Leu	Asn	Gln	Asn	Ile	Val	Glu	Gln	Asp	Ile	Leu	Thr		725	730	735
	Ser	Ile	His	Ile	Glu	Glu	His	Glu	Ile	Asp	Ala	Arg	Asp	Thr	Lys	Leu		740	745	750
55	Gly	Ala	Glu	Glu	Ile	Thr	Arg	Asp	Ile	Pro	Asn	Val	Ser	Glu	Glu	Val		755	760	765

Leu Lys Asp Leu Asp Asp Arg Gly Ile Val Arg Ile Gly Ala Asp Val
 770 775 780
 5 Arg Asp Gly Asp Ile Leu Val Gly Lys Val Thr Pro Lys Gly Glu Thr
 785 790 795 800
 Glu Leu Thr Pro Glu Glu Arg Leu Leu Arg Ala Ile Phe Gly Glu Lys
 805 810 815
 10 Ala Arg Glu Val Arg Asp Thr Ser Met Lys Val Pro His Gly Glu Thr
 820 825 830
 Gly Lys Val Ile Gly Val Arg His Phe Ser Arg Glu Asp Asp Asp Asp
 835 840 845
 15 Leu Ala Pro Gly Val Asn Glu Met Ile Arg Ile Tyr Val Ala Gln Lys
 850 855 860
 20 Arg Lys Ile Gln Asp Gly Asp Lys Leu Ala Gly Arg His Gly Asn Lys
 865 870 875 880
 Gly Val Val Gly Lys Ile Leu Pro Gln Glu Asp Met Pro Phe Leu Pro
 885 890 895
 25 Asp Gly Thr Pro Val Asp Ile Ile Leu Asn Thr His Gly Val Pro Arg
 900 905 910
 Arg Met Asn Ile Gly Gln Val Leu Glu Thr His Leu Gly Trp Leu Ala
 915 920 925
 30 Ser Ala Gly Trp Ser Val Asp Pro Glu Asp Pro Glu Asn Ala Glu Leu
 930 935 940
 35 Val Lys Thr Leu Pro Ala Asp Leu Leu Glu Val Pro Ala Gly Ser Leu
 945 950 955 960
 Thr Ala Thr Pro Val Phe Asp Gly Ala Ser Asn Glu Glu Leu Ala Gly
 965 970 975
 40 Leu Leu Ala Asn Ser Arg Pro Asn Arg Asp Gly Asp Val Met Val Asn
 980 985 990
 Ala Asp Gly Lys Ala Thr Leu Ile Asp Gly Arg Ser Gly Glu Pro Tyr
 995 1000 1005
 45 Pro Tyr Pro Val Ser Ile Gly Tyr Met Tyr Met Leu Lys Leu His His
 1010 1015 1020
 50 Leu Val Asp Glu Lys Ile His Ala Arg Ser Thr Gly Pro Tyr Ser Met
 025 1030 1035 1040
 Ile Thr Gln Gln Pro Leu Gly Gly Lys Ala Gln Phe Gly Gly Gln Arg
 1045 1050 1055
 55 Phe Gly Glu Met Glu Val Trp Ala Met Gln Ala Tyr Gly Ala Ala Tyr
 1060 1065 1070
 Thr Leu Gln Glu Leu Leu Thr Ile Lys Ser Asp Asp Val Val Gly Arg
 1075 1080 1085

Val Lys Val Tyr Glu Ala Ile Val Lys Gly Glu Asn Ile Pro Asp Pro
 1090 1095 1100
 5 Gly Ile Pro Glu Ser Phe Lys Val Leu Leu Lys Glu Leu Gln Ser Leu
 105 1110 1115 1120
 Cys Leu Asn Val Glu Val Leu Ser Ala Asp Gly Thr Pro Met Glu Leu
 1125 1130 1135
 10 Ala Gly Asp Asp Asp Asp Phe Asp Gln Ala Gly Ala Ser Leu Gly Ile
 1140 1145 1150
 Asn Leu Ser Arg Asp Glu Arg Ser Asp Ala Asp Thr Ala
 1155 1160 1165
 15
 20 <210> 3
 <211> 5099
 <212> DNA
 <213> Corynebacterium glutamicum
 <220>
 25 <221> CDS
 <222> (702)..(4196)
 <223> rpoB-Gen Allel 1547
 <400> 3
 30 acaatgtgac tcgtgatttt tgggtggatc agcgtaccgg tttggttgtc gatctagctg 60
 aaaatattga tgattttttac ggcgaccgca gcggccagaa gtacgaacag aaattgcttt 120
 tcgacgcctc cctcgacgat gcagctgtct ctaagctggg tgcacaggcc gaaagcatcc 180
 35 ctgatggaga tgtgagcaaa atcgcaaata ccgtaggtat tgtgatcggg gcggtattgg 240
 ctctcgtggg cctggccggg tgttttgggg cgtttgggaa gaaacgtcga gaagcttaac 300
 40 ctgctgttca aatagatttt ccctgtttcg aattgcggaa accccggggt tgtttgctag 360
 ggtgcctcgt agaaggggtc aagaagattt ctgggaaacg cgcccgtgcg gttggttgct 420
 aatagcacgc ggagcaccag atgaaaaatc tcccctttac tttcgcgcgc gattggtata 480
 45 ctctgagtcg ttgcgttgga attcgtgact ctttttcggt cctgtagcgc caagaccttg 540
 atcaagggtg tttaaaaaaa ccgatttgac aaggtcattc agtgctatct ggagtcgttc 600
 50 aggggggatcg ggttcctcag cagaccaatt gctcaaaaat accagcgggtg ttgatctgca 660
 cttaatggcc ttgaccagcc aggtgcaatt acccgctga g gtg ctg gaa gga ctc 716
 Met Leu Glu Gly Leu
 1 5
 55 atc ttg gca gtc tcc cgc cag acc aag tca gtc gtc gat att ccc ggt 764
 Ile Leu Ala Val Ser Arg Gln Thr Lys Ser Val Val Asp Ile Pro Gly
 10 15 20

		gca	ccg	cag	cgt	tat	tct	ttc	gcg	aag	gtg	tcc	gca	ccc	att	gag	gtg	812
		Ala	Pro	Gln	Arg	Tyr	Ser	Phe	Ala	Lys	Val	Ser	Ala	Pro	Ile	Glu	Val	
					25					30					35			
5		ccc	ggg	cta	cta	gat	ctt	caa	ctg	gat	tct	tac	tcc	tgg	ctg	att	ggt	860
		Pro	Gly	Leu	Leu	Asp	Leu	Gln	Leu	Asp	Ser	Tyr	Ser	Trp	Leu	Ile	Gly	
				40					45					50				
10		acg	cct	gag	tgg	cgt	gct	cgt	cag	aag	gaa	gaa	ttc	ggc	gag	gga	gcc	908
		Thr	Pro	Glu	Trp	Arg	Ala	Arg	Gln	Lys	Glu	Glu	Phe	Gly	Glu	Gly	Ala	
			55					60					65					
15		cgc	gta	acc	agc	ggc	ctt	gag	aac	att	ctc	gag	gag	ctc	tcc	cca	atc	956
		Arg	Val	Thr	Ser	Gly	Leu	Glu	Asn	Ile	Leu	Glu	Glu	Leu	Ser	Pro	Ile	
		70					75					80					85	
20		cag	gat	tac	tct	gga	aac	atg	tcc	ctg	agc	ctt	tcg	gag	cca	cgc	ttc	1004
		Gln	Asp	Tyr	Ser	Gly	Asn	Met	Ser	Leu	Ser	Leu	Ser	Glu	Pro	Arg	Phe	
						90					95					100		
		gaa	gac	gtc	aag	aac	acc	att	gac	gag	gcg	aaa	gaa	aag	gac	atc	aac	1052
		Glu	Asp	Val	Lys	Asn	Thr	Ile	Asp	Glu	Ala	Lys	Glu	Lys	Asp	Ile	Asn	
					105					110					115			
25		tac	gcg	gcg	cca	ctg	tat	gtg	acc	gcg	gag	ttc	gtc	aac	aac	acc	acc	1100
		Tyr	Ala	Ala	Pro	Leu	Tyr	Val	Thr	Ala	Glu	Phe	Val	Asn	Asn	Thr	Thr	
					120				125					130				
30		ggt	gaa	atc	aag	tct	cag	act	gtc	ttc	atc	ggc	gat	ttc	cca	atg	atg	1148
		Gly	Glu	Ile	Lys	Ser	Gln	Thr	Val	Phe	Ile	Gly	Asp	Phe	Pro	Met	Met	
			135					140					145					
35		acg	gac	aag	gga	acg	ttc	atc	atc	aac	gga	acc	gaa	cgc	gtt	gtg	gtc	1196
		Thr	Asp	Lys	Gly	Thr	Phe	Ile	Ile	Asn	Gly	Thr	Glu	Arg	Val	Val	Val	
		150					155					160					165	
40		agc	cag	ctc	gtc	cgc	tcc	ccg	ggc	gtg	tac	ttt	gac	cag	acc	atc	gat	1244
		Ser	Gln	Leu	Val	Arg	Ser	Pro	Gly	Val	Tyr	Phe	Asp	Gln	Thr	Ile	Asp	
						170					175					180		
		aag	tca	act	gag	cgt	cca	ctg	cac	gcc	gtg	aag	gtt	att	cct	ttc	cgt	1292
		Lys	Ser	Thr	Glu	Arg	Pro	Leu	His	Ala	Val	Lys	Val	Ile	Pro	Phe	Arg	
					185					190					195			
45		ggt	gct	tgg	ctt	gag	ttt	gac	gtc	gat	aag	cgc	gat	tcg	gtt	ggt	gtt	1340
		Gly	Ala	Trp	Leu	Glu	Phe	Asp	Val	Asp	Lys	Arg	Asp	Ser	Val	Gly	Val	
				200					205						210			
50		cgt	att	gac	cgc	aag	cgt	cgc	cag	cca	gtc	acc	gta	ctg	ctg	aag	gct	1388
		Arg	Ile	Asp	Arg	Lys	Arg	Arg	Gln	Pro	Val	Thr	Val	Leu	Leu	Lys	Ala	
			215					220					225					
55		ctt	ggc	tgg	acc	act	gag	cag	atc	acc	gag	cgt	ttc	ggt	ttc	tct	gaa	1436
		Leu	Gly	Trp	Thr	Thr	Glu	Gln	Ile	Thr	Glu	Arg	Phe	Gly	Phe	Ser	Glu	
		230					235					240					245	
		atc	atg	atg	tcc	acc	ctc	gag	tcc	gat	ggt	gta	gca	aac	acc	gat	gag	1484
		Ile	Met	Met	Ser	Thr	Leu	Glu	Ser	Asp	Gly	Val	Ala	Asn	Thr	Asp	Glu	
					250						255					260		

5	gca	ttg	ctg	gag	atc	tac	cgc	aag	cag	cgt	cca	ggc	gag	cag	cct	acc	1532
	Ala	Leu	Leu	Glu	Ile	Tyr	Arg	Lys	Gln	Arg	Pro	Gly	Glu	Gln	Pro	Thr	
				265				270							275		
	cgc	gac	ctt	gcg	cag	tcc	ctc	ctg	gac	aac	agc	ttc	ttc	cgt	gca	aag	1580
	Arg	Asp	Leu	Ala	Gln	Ser	Leu	Leu	Asp	Asn	Ser	Phe	Phe	Arg	Ala	Lys	
				280				285							290		
10	cgc	tac	gac	ctg	gct	cgc	gtt	ggc	cgt	tac	aag	atc	aac	cgc	aag	ctc	1628
	Arg	Tyr	Asp	Leu	Ala	Arg	Val	Gly	Arg	Tyr	Lys	Ile	Asn	Arg	Lys	Leu	
				295				300							305		
15	ggc	ctt	ggc	ggc	gac	cac	gat	ggc	ttg	atg	act	ctt	act	gaa	gag	gac	1676
	Gly	Leu	Gly	Gly	Asp	His	Asp	Gly	Leu	Met	Thr	Leu	Thr	Glu	Glu	Asp	
				310				315							320		
20	atc	gca	acc	acc	atc	gag	tac	ctg	gtg	cgt	ctg	cac	gca	ggc	gag	cgc	1724
	Ile	Ala	Thr	Thr	Ile	Glu	Tyr	Leu	Val	Arg	Leu	His	Ala	Gly	Glu	Arg	
				330				335							340		
	gtc	atg	act	tct	cca	aat	ggc	gaa	gag	atc	cca	gtc	gag	acc	gat	gac	1772
	Val	Met	Thr	Ser	Pro	Asn	Gly	Glu	Glu	Ile	Pro	Val	Glu	Thr	Asp	Asp	
				345				350							355		
	atc	gac	cac	ttt	ggc	aac	cgt	cgt	ctg	cgt	acc	gtt	ggc	gaa	ctg	atc	1820
	Ile	Asp	His	Phe	Gly	Asn	Arg	Arg	Leu	Arg	Thr	Val	Gly	Glu	Leu	Ile	
				360				365							370		
30	cag	aac	cag	gtc	cgt	gtc	ggc	ctg	tcc	cgc	atg	gag	cgc	gtt	gtt	cgt	1868
	Gln	Asn	Gln	Val	Arg	Val	Gly	Leu	Ser	Arg	Met	Glu	Arg	Val	Val	Arg	
				375				380							385		
35	gag	cgt	atg	acc	acc	cag	gat	gcg	gag	tcc	att	act	cct	act	tcc	ttg	1916
	Glu	Arg	Met	Thr	Thr	Gln	Asp	Ala	Glu	Ser	Ile	Thr	Pro	Thr	Ser	Leu	
				390				395							400		
40	atc	aac	gtt	cgt	cct	gtc	tct	gca	gct	atc	cgt	gag	ttc	ttc	gga	act	1964
	Ile	Asn	Val	Arg	Pro	Val	Ser	Ala	Ala	Ile	Arg	Glu	Phe	Phe	Gly	Thr	
				410				415							420		
	tcc	cag	ctg	tct	cag	ttc	atg	gtc	cag	aac	aac	tcc	ctg	tct	ggc	ttg	2012
	Ser	Gln	Leu	Ser	Gln	Phe	Met	Val	Gln	Asn	Asn	Ser	Leu	Ser	Gly	Leu	
				425				430							435		
45	act	cac	aag	cgt	cgt	ctg	tcg	gct	ctg	ggc	ccg	ggc	ggc	ctg	tcc	cgt	2060
	Thr	His	Lys	Arg	Arg	Leu	Ser	Ala	Leu	Gly	Pro	Gly	Gly	Leu	Ser	Arg	
				440				445							450		
50	gag	cgc	gcc	ggc	atc	gag	gtt	cga	gac	gtt	cac	cca	tct	cac	tac	ggc	2108
	Glu	Arg	Ala	Gly	Ile	Glu	Val	Arg	Asp	Val	His	Pro	Ser	His	Tyr	Gly	
				455				460							465		
55	cgt	atg	tgc	cca	att	gag	act	ccg	gaa	ggc	cca	aac	att	ggc	ctg	atc	2156
	Arg	Met	Cys	Pro	Ile	Glu	Thr										

		ggt tcc ttg gct tcc tat gct cga gtg aac cca ttc ggt ttc att gag	2204
		Gly Ser Leu Ala Ser Tyr Ala Arg Val Asn Pro Phe Gly Phe Ile Glu	
		490 495 500	
5		acc cca tac cgt cgc atc atc gac ggc aag ctg acc gac cag att gac	2252
		Thr Pro Tyr Arg Arg Ile Ile Asp Gly Lys Leu Thr Asp Gln Ile Asp	
		505 510 515	
10		tac ctt acc gct gat gag gaa gac cgc ttc gtt gtt gcg cag gca aac	2300
		Tyr Leu Thr Ala Asp Glu Glu Asp Arg Phe Val Val Ala Gln Ala Asn	
		520 525 530	
15		acg cac tac gac gaa gag ggc aac atc acc gat gag acc gtc act gtt	2348
		Thr His Tyr Asp Glu Glu Gly Asn Ile Thr Asp Glu Thr Val Thr Val	
		535 540 545	
20		cgt ctg aag gac ggc gac atc gcc atg gtt ggc cgc aac gcg gtt gat	2396
		Arg Leu Lys Asp Gly Asp Ile Ala Met Val Gly Arg Asn Ala Val Asp	
		550 555 560 565	
20		tac atg gac gtt tcc cct cgt cag atg gtt tct gtt ggt acc gcg atg	2444
		Tyr Met Asp Val Ser Pro Arg Gln Met Val Ser Val Gly Thr Ala Met	
		570 575 580	
25		att cca ttc ctg gag cac gac gat gct aac cgt gca ctg atg ggc gcg	2492
		Ile Pro Phe Leu Glu His Asp Asp Ala Asn Arg Ala Leu Met Gly Ala	
		585 590 595	
30		aac atg cag aag cag gct gtg cca ctg att cgt gcc gag gct cct ttc	2540
		Asn Met Gln Lys Gln Ala Val Pro Leu Ile Arg Ala Glu Ala Pro Phe	
		600 605 610	
35		gtg ggc acc ggt atg gag cag cgc gca gca tac gac gcc ggc gac ctg	2588
		Val Gly Thr Gly Met Glu Gln Arg Ala Ala Tyr Asp Ala Gly Asp Leu	
		615 620 625	
40		gtt att acc cca gtc gca ggt gtg gtg gaa aac gtt tca gct gac ttc	2636
		Val Ile Thr Pro Val Ala Gly Val Val Glu Asn Val Ser Ala Asp Phe	
		630 635 640 645	
40		atc acc atc atg gct gat gac ggc aag cgc gaa acc tac ctg ctg cgt	2684
		Ile Thr Ile Met Ala Asp Asp Gly Lys Arg Glu Thr Tyr Leu Leu Arg	
		650 655 660	
45		aag ttc cag cgc acc aac cag ggc acc agc tac aac cag aag cct ttg	2732
		Lys Phe Gln Arg Thr Asn Gln Gly Thr Ser Tyr Asn Gln Lys Pro Leu	
		665 670 675	
50		gtt aac ttg ggc gag cgc gtt gaa gct ggc cag gtt att gct gat ggt	2780
		Val Asn Leu Gly Glu Arg Val Glu Ala Gly Gln Val Ile Ala Asp Gly	
		680 685 690	
55		cca ggt acc ttc aat ggt gaa atg tcc ctt ggc cgt aac ctt ctg gtt	2828
		Pro Gly Thr Phe Asn Gly Glu Met Ser Leu Gly Arg Asn Leu Leu Val	
		695 700 705	
		gcg ttc atg cct tgg gaa ggc cac aac tac gag gat gcg atc atc ctc	2876
		Ala Phe Met Pro Trp Glu Gly His Asn Tyr Glu Asp Ala Ile Ile Leu	
		710 715 720 725	

5	aac cag aac atc gtt gag cag gac atc ttg acc tcg atc cac atc gag	2924
	Asn Gln Asn Ile Val Glu Gln Asp Ile Leu Thr Ser Ile His Ile Glu	
	730 735 740	
10	gag cac gag atc gat gcc cgc gac act aag ctt ggc gcc gaa gaa atc	2972
	Glu His Glu Ile Asp Ala Arg Asp Thr Lys Leu Gly Ala Glu Glu Ile	
	745 750 755	
15	acc cgc gac atc cct aat gtg tct gaa gaa gtc ctc aag gac ctc gac	3020
	Thr Arg Asp Ile Pro Asn Val Ser Glu Glu Val Leu Lys Asp Leu Asp	
	760 765 770	
20	gac cgc ggt att gtc cgc atc ggt gct gat gtt cgt gac ggc gac atc	3068
	Asp Arg Gly Ile Val Arg Ile Gly Ala Asp Val Arg Asp Gly Asp Ile	
	775 780 785	
25	ctg gtc ggt aag gtc acc cct aag ggc gag acc gag ctc acc ccg gaa	3116
	Leu Val Gly Lys Val Thr Pro Lys Gly Glu Thr Glu Leu Thr Pro Glu	
	790 795 800 805	
30	gag cgc ttg ctg cgc gca atc ttc ggt gag aag gcc cgc gaa gtt cgc	3164
	Glu Arg Leu Leu Arg Ala Ile Phe Gly Glu Lys Ala Arg Glu Val Arg	
	810 815 820	
35	gat acc tcc atg aag gtg cct cac ggt gag acc ggc aag gtc atc ggc	3212
	Asp Thr Ser Met Lys Val Pro His Gly Glu Thr Gly Lys Val Ile Gly	
	825 830 835	
40	gtg cgt cac ttc tcc cgc gag gac gac gac gat ctg gct cct ggc gtc	3260
	Val Arg His Phe Ser Arg Glu Asp Asp Asp Asp Leu Ala Pro Gly Val	
	840 845 850	
45	aac gag atg atc cgt atc tac gtt gct cag aag cgt aag atc cag gac	3308
	Asn Glu Met Ile Arg Ile Tyr Val Ala Gln Lys Arg Lys Ile Gln Asp	
	855 860 865	
50	ggc gat aag ctc gct ggc cgc cac ggt aac aag ggt gtt gtc ggt aaa	3356
	Gly Asp Lys Leu Ala Gly Arg His Gly Asn Lys Gly Val Val Gly Lys	
	870 875 880 885	
55	att ttg cct cag gaa gat atg cca ttc ctt cca gac ggc act cct gtt	3404
	Ile Leu Pro Gln Glu Asp Met Pro Phe Leu Pro Asp Gly Thr Pro Val	
	890 895 900	
60	gac atc atc ttg aac acc cac ggt gtt cca cgt cgt atg aac att ggt	3452
	Asp Ile Ile Leu Asn Thr His Gly Val Pro Arg Arg Met Asn Ile Gly	
	905 910 915	
65	cag gtt ctt gag acc cac ctt ggc tgg ctg gca tct gct ggt tgg tcc	3500
	Gln Val Leu Glu Thr His Leu Gly Trp Leu Ala Ser Ala Gly Trp Ser	
	920 925 930	
70	gtg gat cct gaa gat cct gag aac gct gag ctc gtc aag act ctg cct	3548
	Val Asp Pro Glu Asp Pro Glu Asn Ala Glu Leu Val Lys Thr Leu Pro	
	935 940 945	

	gca gac ctc ctc gag gtt cct gct ggt tcc ttg act gca act cct gtg	3596
	Ala Asp Leu Leu Glu Val Pro Ala Gly Ser Leu Thr Ala Thr Pro Val	
	950 955 960 965	
5	ttc gac ggt gcg tca aac gaa gag ctc gca ggc ctg ctc gct aat tca	3644
	Phe Asp Gly Ala Ser Asn Glu Glu Leu Ala Gly Leu Leu Ala Asn Ser	
	970 975 980	
10	cgt cca aac cgc gac ggc gac gtc atg gtt aac gcg gat ggt aaa gca	3692
	Arg Pro Asn Arg Asp Gly Asp Val Met Val Asn Ala Asp Gly Lys Ala	
	985 990 995	
15	acg ctt atc gac ggt cgc tcc ggt gag cct tac ccg tac ccg gtt tcc	3740
	Thr Leu Ile Asp Gly Arg Ser Gly Glu Pro Tyr Pro Tyr Pro Val Ser	
	1000 1005 1010	
20	atc ggc tac atg tac atg ctg aag ctg cac cac ctc gtt gac gag aag	3788
	Ile Gly Tyr Met Tyr Met Leu Lys Leu His His Leu Val Asp Glu Lys	
	1015 1020 1025	
	atc cac gca cgt tcc act ggt cct tac tcc atg att acc cag cag cca	3836
	Ile His Ala Arg Ser Thr Gly Pro Tyr Ser Met Ile Thr Gln Gln Pro	
	1030 1035 1040 1045	
25	ctg ggt ggt aaa gca cag ttc ggt gga cag cgt ttc ggc gaa atg gag	3884
	Leu Gly Gly Lys Ala Gln Phe Gly Gly Gln Arg Phe Gly Glu Met Glu	
	1050 1055 1060	
30	gtg tgg gca atg cag gca tac ggc gct gcc tac aca ctt cag gag ctg	3932
	Val Trp Ala Met Gln Ala Tyr Gly Ala Ala Tyr Thr Leu Gln Glu Leu	
	1065 1070 1075	
35	ctg acc atc aag tct gat gac gtg gtt ggc cgt gtc aag gtc tac gaa	3980
	Leu Thr Ile Lys Ser Asp Asp Val Val Gly Arg Val Lys Val Tyr Glu	
	1080 1085 1090	
40	gca att gtg aag ggc gag aac atc ccg gat cca ggt att cct gag tcc	4028
	Ala Ile Val Lys Gly Glu Asn Ile Pro Asp Pro Gly Ile Pro Glu Ser	
	1095 1100 1105	
	ttc aag gtt ctc ctc aag gag ctc cag tcc ttg tgc ctg aac gtg gag	4076
	Phe Lys Val Leu Leu Lys Glu Leu Gln Ser Leu Cys Leu Asn Val Glu	
	1110 1115 1120 1125	
45	gtt ctc tcc gca gac ggc act cca atg gag ctc gcg ggt gac gac gac	4124
	Val Leu Ser Ala Asp Gly Thr Pro Met Glu Leu Ala Gly Asp Asp Asp	
	1130 1135 1140	
50	gac ttc gat cag gca ggc gcc tca ctt ggc atc aac ctg tcc cgt gac	4172
	Asp Phe Asp Gln Ala Gly Ala Ser Leu Gly Ile Asn Leu Ser Arg Asp	
	1145 1150 1155	
55	gag cgt tcc gac gcc gac acc gca tagcagatca gaaaacaacc gctagaaatc	4226
	Glu Arg Ser Asp Ala Asp Thr Ala	
	1160 1165	
	aagccataca tcccccgac attgaagaga tggtctgggg ggaaaggag ttttacgtgc	4286
	tcgacgtaaa cgtcttcgat gagctccgca tcggcctggc caccgccgac gacatccgcc	4346

gttggtccaa ggggtgaggtc aagaagccgg agaccatcaa ctaccgaacc ctcaagcctg 4406
 5 agaaggacgg tctgttctgc gagcgtatct tcggtccaac tcgcgactgg gagtgcgcct 4466
 gcggtaagta caagcgtgtc cgctacaagg gcatcatctg tgaacgctgt ggcgttgagg 4526
 tcaccaagtc caaggtgcgc cgtgagcgca tgggacacat tgagctcgct gcaccagtaa 4586
 10 cccacatttg gtacttcaag ggcgttccat cagcctcgg ctaccttttg gaccttgctc 4646
 caaaggacct ggacctcatc atctacttcg gtgcgaacat catcaccagc gtggacgaag 4706
 15 aggctcgcca cagcgaccag accactcttg aggcagaaat gcttctggag aagaaggacg 4766
 ttgaggcaga cgcagagtct gacattgctg agcgtgctga aaagctcgaa gaggatcttg 4826
 ctgaacttga ggcagctggc gctaaggccg acgctcgccg caaggttcag gctgctgccg 4886
 20 ataaggaaat gcagcacatc cgtgagcgtg cacagcgca aatcgatcgt ctcgatgagg 4946
 tctggcagac cttcatcaag cttgctccaa agcagatgat ccgcgatgag aagctctacg 5006
 atgaactgat cgaccgctac gaggattact tcaccggtgg tatgggtgca gagtccattg 5066
 25 aggctttgat ccagaacttc gaccttgatg ctg 5099

30 <210> 4
 <211> 1165
 <212> PRT
 <213> Corynebacterium glutamicum

35 <400> 4
 Met Leu Glu Gly Leu Ile Leu Ala Val Ser Arg Gln Thr Lys Ser Val
 1 5 10 15
 Val Asp Ile Pro Gly Ala Pro Gln Arg Tyr Ser Phe Ala Lys Val Ser
 20 25 30
 40 Ala Pro Ile Glu Val Pro Gly Leu Leu Asp Leu Gln Leu Asp Ser Tyr
 35 40 45
 45 Ser Trp Leu Ile Gly Thr Pro Glu Trp Arg Ala Arg Gln Lys Glu Glu
 50 55 60
 Phe Gly Glu Gly Ala Arg Val Thr Ser Gly Leu Glu Asn Ile Leu Glu
 65 70 75 80
 50 Glu Leu Ser Pro Ile Gln Asp Tyr Ser Gly Asn Met Ser Leu Ser Leu
 85 90 95
 Ser Glu Pro Arg Phe Glu Asp Val Lys Asn Thr Ile Asp Glu Ala Lys
 100 105 110
 55 Glu Lys Asp Ile Asn Tyr Ala Ala Pro Leu Tyr Val Thr Ala Glu Phe
 115 120 125

	Val	Asn	Asn	Thr	Thr	Gly	Glu	Ile	Lys	Ser	Gln	Thr	Val	Phe	Ile	Gly	
	130						135					140					
5	Asp	Phe	Pro	Met	Met	Thr	Asp	Lys	Gly	Thr	Phe	Ile	Ile	Asn	Gly	Thr	
	145					150					155					160	
	Glu	Arg	Val	Val	Val	Ser	Gln	Leu	Val	Arg	Ser	Pro	Gly	Val	Tyr	Phe	
					165					170					175		
10	Asp	Gln	Thr	Ile	Asp	Lys	Ser	Thr	Glu	Arg	Pro	Leu	His	Ala	Val	Lys	
				180					185					190			
	Val	Ile	Pro	<u>Phe</u>	Arg	Gly	Ala	Trp	Leu	Glu	Phe	Asp	Val	Asp	Lys	Arg	
			195					200					205				
15	Asp	Ser	Val	Gly	Val	Arg	Ile	Asp	Arg	Lys	Arg	Arg	Gln	Pro	Val	Thr	
		210					215					220					
20	Val	Leu	Leu	Lys	Ala	Leu	Gly	Trp	Thr	Thr	Glu	Gln	Ile	Thr	Glu	Arg	
	225					230					235					240	
	Phe	Gly	Phe	Ser	Glu	Ile	Met	Met	Ser	Thr	Leu	Glu	Ser	Asp	Gly	Val	
					245					250					255		
25	Ala	Asn	Thr	Asp	Glu	Ala	Leu	Leu	Glu	Ile	Tyr	Arg	Lys	Gln	Arg	Pro	
				260					265					270			
	Gly	Glu	Gln	Pro	Thr	Arg	Asp	Leu	Ala	Gln	Ser	Leu	Leu	Asp	Asn	Ser	
			275					280					285				
30	Phe	Phe	Arg	Ala	Lys	Arg	Tyr	Asp	Leu	Ala	Arg	Val	Gly	Arg	Tyr	Lys	
		290					295					300					
35	Ile	Asn	Arg	Lys	Leu	Gly	Leu	Gly	Gly	Asp	His	Asp	Gly	Leu	Met	Thr	
	305					310					315					320	
	Leu	Thr	Glu	Glu	Asp	Ile	Ala	Thr	Thr	Ile	Glu	Tyr	Leu	Val	Arg	Leu	
					325					330					335		
40	His	Ala	Gly	Glu	Arg	Val	Met	Thr	Ser	Pro	Asn	Gly	Glu	Glu	Ile	Pro	
				340					345					350			
	Val	Glu	Thr	Asp	Asp	Ile	Asp	His	Phe	Gly	Asn	Arg	Arg	Leu	Arg	Thr	
			355					360					365				
45	Val	Gly	Glu	Leu	Ile	Gln	Asn	Gln	Val	Arg	Val	Gly	Leu	Ser	Arg	Met	
		370					375					380					
50	Glu	Arg	Val	Val	Arg	Glu	Arg	Met	Thr	Thr	Gln	Asp	Ala	Glu	Ser	Ile	
	385					390					395					400	
	Thr	Pro	Thr	Ser	Leu	Ile	Asn	Val	Arg	Pro	Val	Ser	Ala	Ala	Ile	Arg	
					405					410					415		
55	Glu	Phe	Phe	Gly	Thr	Ser	Gln	Leu	Ser	Gln	Phe	Met	<u>Val</u>	Gln	Asn	Asn	
				420					425					430			
	Ser	Leu	Ser	Gly	Leu	Thr	His	Lys	Arg	Arg	Leu	Ser	Ala	Leu	Gly	Pro	
				435				440					445				

Gly Gly Leu Ser Arg Glu Arg Ala Gly Ile Glu Val Arg Asp Val His
 450 455 460
 5 Pro Ser His Tyr Gly Arg Met Cys Pro Ile Glu Thr Pro Glu Gly Pro
 465 470 475 480
 Asn Ile Gly Leu Ile Gly Ser Leu Ala Ser Tyr Ala Arg Val Asn Pro
 485 490 495
 10 Phe Gly Phe Ile Glu Thr Pro Tyr Arg Arg Ile Ile Asp Gly Lys Leu
 500 505 510
 Thr Asp Gln Ile Asp Tyr Leu Thr Ala Asp Glu Glu Asp Arg Phe Val
 515 520 525
 15 Val Ala Gln Ala Asn Thr His Tyr Asp Glu Glu Gly Asn Ile Thr Asp
 530 535 540
 20 Glu Thr Val Thr Val Arg Leu Lys Asp Gly Asp Ile Ala Met Val Gly
 545 550 555 560
 Arg Asn Ala Val Asp Tyr Met Asp Val Ser Pro Arg Gln Met Val Ser
 565 570 575
 25 Val Gly Thr Ala Met Ile Pro Phe Leu Glu His Asp Asp Ala Asn Arg
 580 585 590
 30 Ala Leu Met Gly Ala Asn Met Gln Lys Gln Ala Val Pro Leu Ile Arg
 595 600 605
 Ala Glu Ala Pro Phe Val Gly Thr Gly Met Glu Gln Arg Ala Ala Tyr
 610 615 620
 35 Asp Ala Gly Asp Leu Val Ile Thr Pro Val Ala Gly Val Val Glu Asn
 625 630 635 640
 Val Ser Ala Asp Phe Ile Thr Ile Met Ala Asp Asp Gly Lys Arg Glu
 645 650 655
 40 Thr Tyr Leu Leu Arg Lys Phe Gln Arg Thr Asn Gln Gly Thr Ser Tyr
 660 665 670
 45 Asn Gln Lys Pro Leu Val Asn Leu Gly Glu Arg Val Glu Ala Gly Gln
 675 680 685
 Val Ile Ala Asp Gly Pro Gly Thr Phe Asn Gly Glu Met Ser Leu Gly
 690 695 700
 50 Arg Asn Leu Leu Val Ala Phe Met Pro Trp Glu Gly His Asn Tyr Glu
 705 710 715 720
 Asp Ala Ile Ile Leu Asn Gln Asn Ile Val Glu Gln Asp Ile Leu Thr
 725 730 735
 55 Ser Ile His Ile Glu Glu His Glu Ile Asp Ala Arg Asp Thr Lys Leu
 740 745 750

Gly Ala Glu Glu Ile Thr Arg Asp Ile Pro Asn Val Ser Glu Glu Val
 755 760 765
 5 Leu Lys Asp Leu Asp Asp Arg Gly Ile Val Arg Ile Gly Ala Asp Val
 770 775 780
 Arg Asp Gly Asp Ile Leu Val Gly Lys Val Thr Pro Lys Gly Glu Thr
 785 790 795 800
 10 Glu Leu Thr Pro Glu Glu Arg Leu Leu Arg Ala Ile Phe Gly Glu Lys
 805 810 815
 Ala Arg Glu Val Arg Asp Thr Ser Met Lys Val Pro His Gly Glu Thr
 820 825 830
 15 Gly Lys Val Ile Gly Val Arg His Phe Ser Arg Glu Asp Asp Asp Asp
 835 840 845
 20 Leu Ala Pro Gly Val Asn Glu Met Ile Arg Ile Tyr Val Ala Gln Lys
 850 855 860
 Arg Lys Ile Gln Asp Gly Asp Lys Leu Ala Gly Arg His Gly Asn Lys
 865 870 875 880
 25 Gly Val Val Gly Lys Ile Leu Pro Gln Glu Asp Met Pro Phe Leu Pro
 885 890 895
 Asp Gly Thr Pro Val Asp Ile Ile Leu Asn Thr His Gly Val Pro Arg
 900 905 910
 30 Arg Met Asn Ile Gly Gln Val Leu Glu Thr His Leu Gly Trp Leu Ala
 915 920 925
 35 Ser Ala Gly Trp Ser Val Asp Pro Glu Asp Pro Glu Asn Ala Glu Leu
 930 935 940
 Val Lys Thr Leu Pro Ala Asp Leu Leu Glu Val Pro Ala Gly Ser Leu
 945 950 955 960
 40 Thr Ala Thr Pro Val Phe Asp Gly Ala Ser Asn Glu Glu Leu Ala Gly
 965 970 975
 Leu Leu Ala Asn Ser Arg Pro Asn Arg Asp Gly Asp Val Met Val Asn
 980 985 990
 45 Ala Asp Gly Lys Ala Thr Leu Ile Asp Gly Arg Ser Gly Glu Pro Tyr
 995 1000 1005
 50 Pro Tyr Pro Val Ser Ile Gly Tyr Met Tyr Met Leu Lys Leu His His
 1010 1015 1020
 Leu Val Asp Glu Lys Ile His Ala Arg Ser Thr Gly Pro Tyr Ser Met
 1025 1030 1035 1040
 55 Ile Thr Gln Gln Pro Leu Gly Gly Lys Ala Gln Phe Gly Gly Gln Arg
 1045 1050 1055
 Phe Gly Glu Met Glu Val Trp Ala Met Gln Ala Tyr Gly Ala Ala Tyr
 1060 1065 1070

Thr Leu Gln Glu Leu Leu Thr Ile Lys Ser Asp Asp Val Val Gly Arg
 1075 1080 1085
 5 Val Lys Val Tyr Glu Ala Ile Val Lys Gly Glu Asn Ile Pro Asp Pro
 1090 1095 1100
 Gly Ile Pro Glu Ser Phe Lys Val Leu Leu Lys Glu Leu Gln Ser Leu
 105 1110 1115 1120
 10 Cys Leu Asn Val Glu Val Leu Ser Ala Asp Gly Thr Pro Met Glu Leu
 1125 1130 1135
 15 Ala Gly Asp Asp Asp Asp Phe Asp Gln Ala Gly Ala Ser Leu Gly Ile
 1140 1145 1150
 Asn Leu Ser Arg Asp Glu Arg Ser Asp Ala Asp Thr Ala
 1155 1160 1165
 20
 <210> 5
 <211> 5099
 <212> DNA
 25 <213> Corynebacterium glutamicum
 <220>
 <221> CDS
 <222> (702)..(4196)
 30 <223> rpoB-Gen Allel 1546
 <400> 5
 acaatgtgac tcgtgatttt tgggtggatc agcgtaccgg tttggttgtc gatctagctg 60
 35 aaaatattga tgattttttac ggcgaccgca gcggccagaa gtacgaacag aaattgcttt 120
 tcgacgcctc cctcgacgat gcagctgtct ctaagctggt tgcacaggcc gaaagcatcc 180
 40 ctgatggaga tgtgagcaaa atcgcaaata ccgtaggtat tgtgatcggg gcggtattgg 240
 ctctcgtggg cctggccggg tgttttgggg cgtttgggaa gaaacgtcga gaagcttaac 300
 ctgctgttca aatagatttt ccctgtttcg aattgcggaa accccgggtt tgtttgctag 360
 45 ggtgcctcgt agaaggggtc aagaagattt ctgggaaacg cgcccgtgcg gttggttgct 420
 aatagcacgc ggagcaccag atgaaaaatc tcccctttac tttcgcgcg c atttggtata 480
 50 ctctgagtcg ttgcgttgga attcgtgact ctttttcggt cctgtagcgc caagaccttg 540
 atcaagggtg tttaaaaaaa ccgatttgac aaggtcattc agtgctatct ggagtcgttc 600
 agggggatcg ggttcctcag cagaccaatt gctcaaaaat accagcgggtg ttgatctgca 660
 55 cttaatggcc ttgaccagcc aggtgcaatt acccgcgtga g gtg ctg gaa gga ccc 716
 Met Leu Glu Gly Pro
 1 5

	atc ttg gca gtc tcc cgc cag acc aag tca gtc gtc gat att ccc ggt	764
	Ile Leu Ala Val Ser Arg Gln Thr Lys Ser Val Val Asp Ile Pro Gly	
	10 15 20	
5	gca ccg cag cgt tat tct ttc gcg aag gtg tcc gca ccc att gag gtg	812
	Ala Pro Gln Arg Tyr Ser Phe Ala Lys Val Ser Ala Pro Ile Glu Val	
	25 30 35	
10	ccc ggg cta cta gat ctt caa ctg gat tct tac tcc tgg ctg att ggt	860
	Pro Gly Leu Leu Asp Leu Gln Leu Asp Ser Tyr Ser Trp Leu Ile Gly	
	40 45 50	
15	acg cct gag tgg cgt gct cgt cag aag gaa gaa ttc ggc gag gga gcc	908
	Thr Pro Glu Trp Arg Ala Arg Gln Lys Glu Glu Phe Gly Glu Gly Ala	
	55 60 65	
20	cgc gta acc agc ggc ctt gag aac att ctc gag gag ctc tcc cca atc	956
	Arg Val Thr Ser Gly Leu Glu Asn Ile Leu Glu Glu Leu Ser Pro Ile	
	70 75 80 85	
20	cag gat tac tct gga aac atg tcc ctg agc ctt tcg gag cca cgc ttc	1004
	Gln Asp Tyr Ser Gly Asn Met Ser Leu Ser Leu Ser Glu Pro Arg Phe	
	90 95 100	
25	gaa gac gtc aag aac acc att gac gag gcg aaa gaa aag gac atc aac	1052
	Glu Asp Val Lys Asn Thr Ile Asp Glu Ala Lys Glu Lys Asp Ile Asn	
	105 110 115	
30	tac gcg gcg cca ctg tat gtg acc gcg gag ttc gtc aac aac acc acc	1100
	Tyr Ala Ala Pro Leu Tyr Val Thr Ala Glu Phe Val Asn Asn Thr Thr	
	120 125 130	
35	ggt gaa atc aag tct cag act gtc ttc atc ggc gat ttc cca atg atg	1148
	Gly Glu Ile Lys Ser Gln Thr Val Phe Ile Gly Asp Phe Pro Met Met	
	135 140 145	
40	acg gac aag gga acg ttc atc atc aac gga acc gaa cgc gtt gtg gtc	1196
	Thr Asp Lys Gly Thr Phe Ile Ile Asn Gly Thr Glu Arg Val Val Val	
	150 155 160 165	
40	agc cag ctc gtc cgc tcc ccg ggc gtg tac ttt gac cag acc atc gat	1244
	Ser Gln Leu Val Arg Ser Pro Gly Val Tyr Phe Asp Gln Thr Ile Asp	
	170 175 180	
45	aag tca act gag cgt cca ctg cac gcc gtg aag gtt att cct tcc cgt	1292
	Lys Ser Thr Glu Arg Pro Leu His Ala Val Lys Val Ile Pro Ser Arg	
	185 190 195	
50	ggt gct tgg ctt gag ttt gac gtc gat aag cgc gat tcg gtt ggt gtt	1340
	Gly Ala Trp Leu Glu Phe Asp Val Asp Lys Arg Asp Ser Val Gly Val	
	200 205 210	
55	cgt att gac cgc aag cgt cgc cag cca gtc acc gta ctg ctg aag gct	1388
	Arg Ile Asp Arg Lys Arg Arg Gln Pro Val Thr Val Leu Leu Lys Ala	
	215 220 225	
	ctt ggc tgg acc act gag cag atc acc gag cgt ttc ggt ttc tct gaa	1436
	Leu Gly Trp Thr Thr Glu Gln Ile Thr Glu Arg Phe Gly Phe Ser Glu	
	230 235 240 245	

5	atc atg atg tcc acc ctc gag tcc gat ggt gta gca aac acc gat gag	1484
	Ile Met Met Ser Thr Leu Glu Ser Asp Gly Val Ala Asn Thr Asp Glu 250 255 260	
10	gca ttg ctg gag atc tac cgc aag cag cgt cca ggc gag cag cct acc	1532
	Ala Leu Leu Glu Ile Tyr Arg Lys Gln Arg Pro Gly Glu Gln Pro Thr 265 270 275	
15	cgc gac ctt gcg cag tcc ctc ctg gac aac agc ttc ttc cgt gca aag	1580
	Arg Asp Leu Ala Gln Ser Leu Leu Asp Asn Ser Phe Phe Arg Ala Lys 280 285 290	
20	cgc tac gac ctg gct cgc gtt ggt cgt tac aag atc aac cgc aag ctc	1628
	Arg Tyr Asp Leu Ala Arg Val Gly Arg Tyr Lys Ile Asn Arg Lys Leu 295 300 305	
25	ggc ctt ggt ggc gac cac gat ggt ttg atg act ctt act gaa gag gac	1676
	Gly Leu Gly Gly Asp His Asp Gly Leu Met Thr Leu Thr Glu Glu Asp 310 315 320 325	
30	atc gca acc acc atc gag tac ctg gtg cgt ctg cac gca ggt gag cgc	1724
	Ile Ala Thr Thr Ile Glu Tyr Leu Val Arg Leu His Ala Gly Glu Arg 330 335 340	
35	gtc atg act tct cca aat ggt gaa gag atc cca gtc gag acc gat gac	1772
	Val Met Thr Ser Pro Asn Gly Glu Glu Ile Pro Val Glu Thr Asp Asp 345 350 355	
40	atc gac cac ttt ggt aac cgt cgt ctg cgt acc gtt ggc gaa ctg atc	1820
	Ile Asp His Phe Gly Asn Arg Arg Leu Arg Thr Val Gly Glu Leu Ile 360 365 370	
45	cag aac cag gtc cgt gtc ggc ctg tcc cgc atg gag cgc gtt gtt cgt	1868
	Gln Asn Gln Val Arg Val Gly Leu Ser Arg Met Glu Arg Val Val Arg 375 380 385	
50	gag cgt atg acc acc cag gat gcg gag tcc att act cct act tcc ttg	1916
	Glu Arg Met Thr Thr Gln Asp Ala Glu Ser Ile Thr Pro Thr Ser Leu 390 395 400 405	
55	atc aac gtt cgt cct gtc tct gca gct atc cgt gag ttc ttc gga act	1964
	Ile Asn Val Arg Pro Val Ser Ala Ala Ile Arg Glu Phe Phe Gly Thr 410 415 420	
60	tcc cag ctg tct cag ttc atg gac cag aac aac tcc ctg tct ggt ttg	2012
	Ser Gln Leu Ser Gln Phe Met Asp Gln Asn Asn Ser Leu Ser Gly Leu 425 430 435	
65	act tac aag cgt cgt ctg tcg gct ctg ggc ccg ggt ggt ctg tcc cgt	2060
	Thr Tyr Lys Arg Arg Leu Ser Ala Leu Gly Pro Gly Gly Leu Ser Arg 440 445 450	
70	gag cgc gcc ggc atc gag gtt cga gac gtt cac cca tct cac tac ggc	2108
	Glu Arg Ala Gly Ile Glu Val Arg Asp Val His Pro Ser His Tyr Gly 455 460 465	

	cgt atg tgc cca att gag act ccg gaa ggt cca aac att ggc ctg atc	2156
	Arg Met Cys Pro Ile Glu Thr Pro Glu Gly Pro Asn Ile Gly Leu Ile	
	470 475 480 485	
5	ggt tcc ttg gct tcc tat gct cga gtg aac cca ttc ggt ttc att gag	2204
	Gly Ser Leu Ala Ser Tyr Ala Arg Val Asn Pro Phe Gly Phe Ile Glu	
	490 495 500	
10	acc cca tac cgt cgc atc atc gac ggc aag ctg acc gac cag att gac	2252
	Thr Pro Tyr Arg Arg Ile Ile Asp Gly Lys Leu Thr Asp Gln Ile Asp	
	505 510 515	
15	tac ctt acc gct gat gag gaa gac cgc ttc gtt gtt gcg cag gca aac	2300
	Tyr Leu Thr Ala Asp Glu Glu Asp Arg Phe Val Val Ala Gln Ala Asn	
	520 525 530	
20	acg cac tac gac gaa gag ggc aac atc acc gat gag acc gtc act gtt	2348
	Thr His Tyr Asp Glu Glu Gly Asn Ile Thr Asp Glu Thr Val Thr Val	
	535 540 545	
25	cgt ctg aag gac ggc gac atc gcc atg gtt ggc cgc aac gcg gtt gat	2396
	Arg Leu Lys Asp Gly Asp Ile Ala Met Val Gly Arg Asn Ala Val Asp	
	550 555 560 565	
30	tac atg gac gtt tcc cct cgt cag atg gtt tct gtt ggt acc gcg atg	2444
	Tyr Met Asp Val Ser Pro Arg Gln Met Val Ser Val Gly Thr Ala Met	
	570 575 580	
35	att cca ttc ctg gag cac gac gat gct aac cgt gca ctg atg ggc gcg	2492
	Ile Pro Phe Leu Glu His Asp Asp Ala Asn Arg Ala Leu Met Gly Ala	
	585 590 595	
40	aac atg cag aag cag gct gtg cca ctg att cgt gcc gag gct cct ttc	2540
	Asn Met Gln Lys Gln Ala Val Pro Leu Ile Arg Ala Glu Ala Pro Phe	
	600 605 610	
45	gtg ggc acc ggt atg gag cag cgc gca gca tac gac gcc ggc gac ctg	2588
	Val Gly Thr Gly Met Glu Gln Arg Ala Ala Tyr Asp Ala Gly Asp Leu	
	615 620 625	
50	gtt att acc cca gtc gca ggt gtg gtg gaa aac gtt tca gct gac ttc	2636
	Val Ile Thr Pro Val Ala Gly Val Val Glu Asn Val Ser Ala Asp Phe	
	630 635 640 645	
55	atc acc atc atg gct gat gac ggc aag cgc gaa acc tac ctg ctg cgt	2684
	Ile Thr Ile Met Ala Asp Asp Gly Lys Arg Glu Thr Tyr Leu Leu Arg	
	650 655 660	
60	aag ttc cag cgc acc aac cag ggc acc agc tac aac cag aag cct ttg	2732
	Lys Phe Gln Arg Thr Asn Gln Gly Thr Ser Tyr Asn Gln Lys Pro Leu	
	665 670 675	
65	gtt aac ttg ggc gag cgc gtt gaa gct ggc cag gtt att gct gat ggt	2780
	Val Asn Leu Gly Glu Arg Val Glu Ala Gly Gln Val Ile Ala Asp Gly	
	680 685 690	
70	cca ggt acc ttc aat ggt gaa atg tcc ctt ggc cgt aac ctt ctg gtt	2828
	Pro Gly Thr Phe Asn Gly Glu Met Ser Leu Gly Arg Asn Leu Leu Val	
	695 700 705	

5	gcg ttc atg cct tgg gaa ggc cac aac tac gag gat gcg atc atc ctc Ala Phe Met Pro Trp Glu Gly His Asn Tyr Glu Asp Ala Ile Ile Leu 710 715 720 725	2876
10	aac cag aac atc gtt gag cag gac atc ttg acc tcg atc cac atc gag Asn Gln Asn Ile Val Glu Gln Asp Ile Leu Thr Ser Ile His Ile Glu 730 735 740	2924
15	gag cac gag atc gat gcc cgc gac act aag ctt ggc gcc gaa gaa atc Glu His Glu Ile Asp Ala Arg Asp Thr Lys Leu Gly Ala Glu Glu Ile 745 750 755	2972
20	acc cgc gac atc cct aat gtg tct gaa gaa gtc ctc aag gac ctc gac Thr Arg Asp Ile Pro Asn Val Ser Glu Glu Val Leu Lys Asp Leu Asp 760 765 770	3020
25	gac cgc ggt att gtc cgc atc ggt gct gat gtt cgt gac ggc gac atc Asp Arg Gly Ile Val Arg Ile Gly Ala Asp Val Arg Asp Gly Asp Ile 775 780 785	3068
30	ctg gtc ggt aag gtc acc cct aag ggc gag acc gag ctc acc ccg gaa Leu Val Gly Lys Val Thr Pro Lys Gly Glu Thr Glu Leu Thr Pro Glu 790 795 800 805	3116
35	gag cgc ttg ctg cgc gca atc ttc ggt gag aag gcc cgc gaa gtt cgc Glu Arg Leu Leu Arg Ala Ile Phe Gly Glu Lys Ala Arg Glu Val Arg 810 815 820	3164
40	gat acc tcc atg aag gtg cct cac ggt gag acc ggc aag gtc atc ggc Asp Thr Ser Met Lys Val Pro His Gly Glu Thr Gly Lys Val Ile Gly 825 830 835	3212
45	gtg cgt cac ttc tcc cgc gag gac gac gat ctg gct cct ggc gtc Val Arg His Phe Ser Arg Glu Asp Asp Asp Leu Ala Pro Gly Val 840 845 850	3260
50	aac gag atg atc cgt atc tac gtt gct cag aag cgt aag atc cag gac Asn Glu Met Ile Arg Ile Tyr Val Ala Gln Lys Arg Lys Ile Gln Asp 855 860 865	3308
55	ggc gat aag ctc gct ggc cgc cac ggt aac aag ggt gtt gtc ggt aaa Gly Asp Lys Leu Ala Gly Arg His Gly Asn Lys Gly Val Val Gly Lys 870 875 880 885	3356
60	att ttg cct cag gaa gat atg cca ttc ctt cca gac ggc act cct gtt Ile Leu Pro Gln Glu Asp Met Pro Phe Leu Pro Asp Gly Thr Pro Val 890 895 900	3404
65	gac atc atc ttg aac acc cac ggt gtt cca cgt cgt atg aac att ggt Asp Ile Ile Leu Asn Thr His Gly Val Pro Arg Arg Met Asn Ile Gly 905 910 915	3452
70	cag gtt ctt gag acc cac ctt ggc tgg ctg gca tct gct ggt tgg tcc Gln Val Leu Glu Thr His Leu Gly Trp Leu Ala Ser Ala Gly Trp Ser 920 925 930	3500

	gtg gat cct gaa gat cct gag aac gct gag ctc gtc aag act ctg cct	3548
	Val Asp Pro Glu Asp Pro Glu Asn Ala Glu Leu Val Lys Thr Leu Pro	
	935 940 945	
5	gca gac ctc ctc gag gtt cct gct ggt tcc ttg act gca act cct gtg	3596
	Ala Asp Leu Leu Glu Val Pro Ala Gly Ser Leu Thr Ala Thr Pro Val	
	950 955 960 965	
10	ttc gac ggt gcg tca aac gaa gag ctc gca ggc ctg ctc gct aat tca	3644
	Phe Asp Gly Ala Ser Asn Glu Glu Leu Ala Gly Leu Leu Ala Asn Ser	
	970 975 980	
15	cgt cca aac cgc gac ggc gac gtc atg gtt aac gcg gat ggt aaa gca	3692
	Arg Pro Asn Arg Asp Gly Asp Val Met Val Asn Ala Asp Gly Lys Ala	
	985 990 995	
20	acg ctt atc gac ggt cgc tcc ggt gag cct tac ccg tac ccg gtt tcc	3740
	Thr Leu Ile Asp Gly Arg Ser Gly Glu Pro Tyr Pro Tyr Pro Val Ser	
	1000 1005 1010	
	atc ggc tac atg tac atg ctg aag ctg cac cac ctc gtt gac gag aag	3788
	Ile Gly Tyr Met Tyr Met Leu Lys Leu His His Leu Val Asp Glu Lys	
	1015 1020 1025	
25	atc cac gca cgt tcc act ggt cct tac tcc atg att acc cag cag cca	3836
	Ile His Ala Arg Ser Thr Gly Pro Tyr Ser Met Ile Thr Gln Gln Pro	
	1030 1035 1040 1045	
30	ctg ggt ggt aaa gca cag ttc ggt gga cag cgt ttc ggc gaa atg gag	3884
	Leu Gly Gly Lys Ala Gln Phe Gly Gly Gln Arg Phe Gly Glu Met Glu	
	1050 1055 1060	
35	gtg tgg gca atg cag gca tac ggc gct gcc tac aca ctt cag gag ctg	3932
	Val Trp Ala Met Gln Ala Tyr Gly Ala Ala Tyr Thr Leu Gln Glu Leu	
	1065 1070 1075	
40	ctg acc atc aag tct gat gac gtg gtt ggc cgt gtc aag gtc tac gaa	3980
	Leu Thr Ile Lys Ser Asp Asp Val Val Gly Arg Val Lys Val Tyr Glu	
	1080 1085 1090	
	gca att gtg aag ggc gag aac atc ccg gat cca ggt att cct gag tcc	4028
	Ala Ile Val Lys Gly Glu Asn Ile Pro Asp Pro Gly Ile Pro Glu Ser	
	1095 1100 1105	
45	ttc aag gtt ctc ctc aag gag ctc cag tcc ttg tgc ctg aac gtg gag	4076
	Phe Lys Val Leu Leu Lys Glu Leu Gln Ser Leu Cys Leu Asn Val Glu	
	1110 1115 1120 1125	
50	gtt ctc tcc gca gac ggc act cca atg gag ctc gcg ggt gac gac gac	4124
	Val Leu Ser Ala Asp Gly Thr Pro Met Glu Leu Ala Gly Asp Asp Asp	
	1130 1135 1140	
55	gac ttc gat cag gca ggc gcc tca ctt ggc atc aac ctg tcc cgt gac	4172
	Asp Phe Asp Gln Ala Gly Ala Ser Leu Gly Ile Asn Leu Ser Arg Asp	
	1145 1150 1155	
	gag cgt tcc gac gcc gac acc gca tagcagatca gaaaacaacc gctagaaatc	4226
	Glu Arg Ser Asp Ala Asp Thr Ala	
	1160 1165	

aagccataca tcccccgac attgaagaga tgttctggg ggaaaggag ttttacgtgc 4286
 5 tgcacgtaaa cgtcttcgat gagctccgca tcggcctggc caccgccgac gacatccgcc 4346
 gttggtccaa ggggtgaggtc aagaagccgg agaccatcaa ctaccgaacc ctcaagcctg 4406
 agaaggacgg tctgttctgc gagcgtatct tcggtccaac tcgcgactgg gagtgcgctt 4466
 10 gcggtaaagta caagcgtgtc cgctacaagg gcatcatctg tgaacgctgt ggcgttgagg 4526
 tcaccaagtc caaggtgcgc cgtgagcgca tgggacacat tgagctcgct gcaccagtaa 4586
 15 cccacatttg gtacttcaag ggcgttccat cagcctcgg ctaccttttg gaccttgctc 4646
 caaaggacct ggacctcatc atctacttcg gtgcgaacat catcaccagc gtggacgaag 4706
 aggctcgcca cagcgaccag accactcttg aggcagaaat gcttctggag aagaaggacg 4766
 20 ttgaggcaga cgcagagtct gacattgctg agcgtgctga aaagctcgaa gaggatcttg 4826
 ctgaacttga ggcagctggc gctaaggccg acgctcgccg caaggttcag gctgctgccg 4886
 ataaggaaat gcagcacatc cgtgagcgtg cacagcgcca aatcgatcgt ctcgatgagg 4946
 25 tctggcagac cttcatcaag cttgctccaa agcagatgat ccgcatgag aagctctacg 5006
 atgaactgat cgaccgctac gaggattact tcaccggtgg tatgggtgca gagtccattg 5066
 30 aggctttgat ccagaacttc gaccttgatg ctg 5099

<210> 6
 <211> 1165
 35 <212> PRT
 <213> Corynebacterium glutamicum

<400> 6
 40 Met Leu Glu Gly Pro Ile Leu Ala Val Ser Arg Gln Thr Lys Ser Val
 1 5 10 15
 Val Asp Ile Pro Gly Ala Pro Gln Arg Tyr Ser Phe Ala Lys Val Ser
 20 25 30
 45 Ala Pro Ile Glu Val Pro Gly Leu Leu Asp Leu Gln Leu Asp Ser Tyr
 35 40 45
 Ser Trp Leu Ile Gly Thr Pro Glu Trp Arg Ala Arg Gln Lys Glu Glu
 50 55 60
 Phe Gly Glu Gly Ala Arg Val Thr Ser Gly Leu Glu Asn Ile Leu Glu
 65 70 75 80
 55 Glu Leu Ser Pro Ile Gln Asp Tyr Ser Gly Asn Met Ser Leu Ser Leu
 85 90 95
 Ser Glu Pro Arg Phe Glu Asp Val Lys Asn Thr Ile Asp Glu Ala Lys
 100 105 110

Glu Lys Asp Ile Asn Tyr Ala Ala Pro Leu Tyr Val Thr Ala Glu Phe
 115 120 125
 5 Val Asn Asn Thr Thr Gly Glu Ile Lys Ser Gln Thr Val Phe Ile Gly
 130 135 140
 Asp Phe Pro Met Met Thr Asp Lys Gly Thr Phe Ile Ile Asn Gly Thr
 145 150 155 160
 10 Glu Arg Val Val Val Ser Gln Leu Val Arg Ser Pro Gly Val Tyr Phe
 165 170 175
 Asp Gln Thr Ile Asp Lys Ser Thr Glu Arg Pro Leu His Ala Val Lys
 180 185 190
 15 Val Ile Pro Ser Arg Gly Ala Trp Leu Glu Phe Asp Val Asp Lys Arg
 195 200 205
 20 Asp Ser Val Gly Val Arg Ile Asp Arg Lys Arg Arg Gln Pro Val Thr
 210 215 220
 Val Leu Leu Lys Ala Leu Gly Trp Thr Thr Glu Gln Ile Thr Glu Arg
 225 230 235 240
 25 Phe Gly Phe Ser Glu Ile Met Met Ser Thr Leu Glu Ser Asp Gly Val
 245 250 255
 Ala Asn Thr Asp Glu Ala Leu Leu Glu Ile Tyr Arg Lys Gln Arg Pro
 260 265 270
 30 Gly Glu Gln Pro Thr Arg Asp Leu Ala Gln Ser Leu Leu Asp Asn Ser
 275 280 285
 35 Phe Phe Arg Ala Lys Arg Tyr Asp Leu Ala Arg Val Gly Arg Tyr Lys
 290 295 300
 Ile Asn Arg Lys Leu Gly Leu Gly Gly Asp His Asp Gly Leu Met Thr
 305 310 315 320
 40 Leu Thr Glu Glu Asp Ile Ala Thr Thr Ile Glu Tyr Leu Val Arg Leu
 325 330 335
 His Ala Gly Glu Arg Val Met Thr Ser Pro Asn Gly Glu Glu Ile Pro
 340 345 350
 45 Val Glu Thr Asp Asp Ile Asp His Phe Gly Asn Arg Arg Leu Arg Thr
 355 360 365
 50 Val Gly Glu Leu Ile Gln Asn Gln Val Arg Val Gly Leu Ser Arg Met
 370 375 380
 Glu Arg Val Val Arg Glu Arg Met Thr Thr Gln Asp Ala Glu Ser Ile
 385 390 395 400
 55 Thr Pro Thr Ser Leu Ile Asn Val Arg Pro Val Ser Ala Ala Ile Arg
 405 410 415
 Glu Phe Phe Gly Thr Ser Gln Leu Ser Gln Phe Met Asp Gln Asn Asn
 420 425 430

	Ser	Leu	Ser	Gly	Leu	Thr	<u>Tyr</u>	Lys	Arg	Arg	Leu	Ser	Ala	Leu	Gly	Pro
		435						440					445			
5	Gly	Gly	Leu	Ser	Arg	Glu	Arg	Ala	Gly	Ile	Glu	Val	Arg	Asp	Val	His
		450					455					460				
	Pro	Ser	His	Tyr	Gly	Arg	Met	Cys	Pro	Ile	Glu	Thr	Pro	Glu	Gly	Pro
10		465				470					475					480
	Asn	Ile	Gly	Leu	Ile	Gly	Ser	Leu	Ala	Ser	Tyr	Ala	Arg	Val	Asn	Pro
					485					490					495	
15	Phe	Gly	Phe	Ile	Glu	Thr	Pro	Tyr	Arg	Arg	Ile	Ile	Asp	Gly	Lys	Leu
				500					505					510		
	Thr	Asp	Gln	Ile	Asp	Tyr	Leu	Thr	Ala	Asp	Glu	Glu	Asp	Arg	Phe	Val
			515					520					525			
20	Val	Ala	Gln	Ala	Asn	Thr	His	Tyr	Asp	Glu	Glu	Gly	Asn	Ile	Thr	Asp
		530					535					540				
	Glu	Thr	Val	Thr	Val	Arg	Leu	Lys	Asp	Gly	Asp	Ile	Ala	Met	Val	Gly
25		545				550					555					560
	Arg	Asn	Ala	Val	Asp	Tyr	Met	Asp	Val	Ser	Pro	Arg	Gln	Met	Val	Ser
					565					570					575	
30	Val	Gly	Thr	Ala	Met	Ile	Pro	Phe	Leu	Glu	His	Asp	Asp	Ala	Asn	Arg
				580					585					590		
	Ala	Leu	Met	Gly	Ala	Asn	Met	Gln	Lys	Gln	Ala	Val	Pro	Leu	Ile	Arg
			595					600					605			
35	Ala	Glu	Ala	Pro	Phe	Val	Gly	Thr	Gly	Met	Glu	Gln	Arg	Ala	Ala	Tyr
		610					615					620				
	Asp	Ala	Gly	Asp	Leu	Val	Ile	Thr	Pro	Val	Ala	Gly	Val	Val	Glu	Asn
40		625				630					635					640
	Val	Ser	Ala	Asp	Phe	Ile	Thr	Ile	Met	Ala	Asp	Asp	Gly	Lys	Arg	Glu
					645					650					655	
45	Thr	Tyr	Leu	Leu	Arg	Lys	Phe	Gln	Arg	Thr	Asn	Gln	Gly	Thr	Ser	Tyr
				660					665					670		
	Asn	Gln	Lys	Pro	Leu	Val	Asn	Leu	Gly	Glu	Arg	Val	Glu	Ala	Gly	Gln
			675					680					685			
50	Val	Ile	Ala	Asp	Gly	Pro	Gly	Thr	Phe	Asn	Gly	Glu	Met	Ser	Leu	Gly
		690					695					700				
	Arg	Asn	Leu	Leu	Val	Ala	Phe	Met	Pro	Trp	Glu	Gly	His	Asn	Tyr	Glu
55		705				710					715					720
	Asp	Ala	Ile	Ile	Leu	Asn	Gln	Asn	Ile	Val	Glu	Gln	Asp	Ile	Leu	Thr
					725					730					735	

Ser Ile His Ile Glu Glu His Glu Ile Asp Ala Arg Asp Thr Lys Leu
 740 745 750
 5 Gly Ala Glu Glu Ile Thr Arg Asp Ile Pro Asn Val Ser Glu Glu Val
 755 760 765
 Leu Lys Asp Leu Asp Asp Arg Gly Ile Val Arg Ile Gly Ala Asp Val
 770 775 780
 10 Arg Asp Gly Asp Ile Leu Val Gly Lys Val Thr Pro Lys Gly Glu Thr
 785 790 795 800
 Glu Leu Thr Pro Glu Glu Arg Leu Leu Arg Ala Ile Phe Gly Glu Lys
 805 810 815
 15 Ala Arg Glu Val Arg Asp Thr Ser Met Lys Val Pro His Gly Glu Thr
 820 825 830
 Gly Lys Val Ile Gly Val Arg His Phe Ser Arg Glu Asp Asp Asp Asp
 835 840 845
 20 Leu Ala Pro Gly Val Asn Glu Met Ile Arg Ile Tyr Val Ala Gln Lys
 850 855 860
 25 Arg Lys Ile Gln Asp Gly Asp Lys Leu Ala Gly Arg His Gly Asn Lys
 865 870 875 880
 Gly Val Val Gly Lys Ile Leu Pro Gln Glu Asp Met Pro Phe Leu Pro
 885 890 895
 30 Asp Gly Thr Pro Val Asp Ile Ile Leu Asn Thr His Gly Val Pro Arg
 900 905 910
 Arg Met Asn Ile Gly Gln Val Leu Glu Thr His Leu Gly Trp Leu Ala
 915 920 925
 35 Ser Ala Gly Trp Ser Val Asp Pro Glu Asp Pro Glu Asn Ala Glu Leu
 930 935 940
 40 Val Lys Thr Leu Pro Ala Asp Leu Leu Glu Val Pro Ala Gly Ser Leu
 945 950 955 960
 Thr Ala Thr Pro Val Phe Asp Gly Ala Ser Asn Glu Glu Leu Ala Gly
 965 970 975
 45 Leu Leu Ala Asn Ser Arg Pro Asn Arg Asp Gly Asp Val Met Val Asn
 980 985 990
 Ala Asp Gly Lys Ala Thr Leu Ile Asp Gly Arg Ser Gly Glu Pro Tyr
 995 1000 1005
 50 Pro Tyr Pro Val Ser Ile Gly Tyr Met Tyr Met Leu Lys Leu His His
 1010 1015 1020
 Leu Val Asp Glu Lys Ile His Ala Arg Ser Thr Gly Pro Tyr Ser Met
 1025 1030 1035 1040
 Ile Thr Gln Gln Pro Leu Gly Gly Lys Ala Gln Phe Gly Gly Gln Arg
 1045 1050 1055

Phe Gly Glu Met Glu Val Trp Ala Met Gln Ala Tyr Gly Ala Ala Tyr
 1060 1065 1070

5 Thr Leu Gln Glu Leu Leu Thr Ile Lys Ser Asp Asp Val Val Gly Arg
 1075 1080 1085

Val Lys Val Tyr Glu Ala Ile Val Lys Gly Glu Asn Ile Pro Asp Pro
 10 1090 1095 1100

Gly Ile Pro Glu Ser Phe Lys Val Leu Leu Lys Glu Leu Gln Ser Leu
 105 1110 1115 1120

15 Cys Leu Asn Val Glu Val Leu Ser Ala Asp Gly Thr Pro Met Glu Leu
 1125 1130 1135

Ala Gly Asp Asp Asp Asp Phe Asp Gln Ala Gly Ala Ser Leu Gly Ile
 1140 1145 1150

20 Asn Leu Ser Arg Asp Glu Arg Ser Asp Ala Asp Thr Ala
 1155 1160 1165

25 <210> 7
 <211> 1775
 <212> DNA
 <213> Corynebacterium glutamicum

30 <220>
 <221> CDS
 <222> (500)..(880)
 <223> rpsL-Gen

35 <400> 7
 cagctctaca agagtgtcta agtggcgggc attccatgct ttggaggagc gatcttcaaa 60
 ttctctcaaaa gtgagttgac ctcgggaaac agctgcagaa agttcatcca cgacttggtt 120

40 tcggttaagg tcagtggcga gcttctttgc tggttcgttt ccttgaggaa cagtcattggg 180
 aaccattcta acaagggatt tgggtgtttc tgcggctagc tgataatgtg aacggctgag 240

45 tcccactctt gtagttggga attgacggca cctcgcactc aagcgcggta tcgcccctgg 300
 ttttccggga cgcggtggcg catgtttgca tttgatgagg ttgtccgtga catgtttggt 360
 cgggccccaa aaagagcccc cttttttgcg tgtctggaca ctttttcaaa tccttcgcca 420

50 tcgacaagct cagccttcgt gttcgtcccc cgggcgtcac gtcagcagtt aaagaacaac 480
 tccgaaataa ggatggttc atg cca act att cag cag ctg gtc cgt aag ggc 532
 1 5 10
 Met Pro Thr Ile Gln Gln Leu Val Arg Lys Gly

55 cgc cac gat aag tcc gcc aag gtg gct acc gcg gca ctg aag ggt tcc 580
 Arg His Asp Lys Ser Ala Lys Val Ala Thr Ala Ala Leu Lys Gly Ser
 15 20 25

cct cag cgt cgt ggc gta tgc acc cgt gtg tac acc acc acc cct aag 628
 Pro Gln Arg Arg Gly Val Cys Thr Arg Val Tyr Thr Thr Thr Pro Lys
 30 35 40

5 aag cct aac tct gct ctt cgt aag gtc gct cgt gtg cgc ctt acc tcc 676
 Lys Pro Asn Ser Ala Leu Arg Lys Val Ala Arg Val Arg Leu Thr Ser
 45 50 55

10 ggc atc gag gtt tcc gct tac atc cct ggt gag ggc cac aac ctg cag 724
 Gly Ile Glu Val Ser Ala Tyr Ile Pro Gly Glu Gly His Asn Leu Gln
 60 65 70 75

15 gag cac tcc atg gtg ctc gtt cgc ggt ggt cgt gtt aag gac ctc cca 772
 Glu His Ser Met Val Leu Val Arg Gly Gly Arg Val Lys Asp Leu Pro
 80 85 90

20 ggt gtc cgt tac aag atc gtc cgt ggc gca ctg gat acc cag ggt gtt 820
 Gly Val Arg Tyr Lys Ile Val Arg Gly Ala Leu Asp Thr Gln Gly Val
 95 100 105

aag gac cgc aag cag gct cgt tcc ccg cta cgg cgc gaa gag ggg ata 868
 Lys Asp Arg Lys Gln Ala Arg Ser Pro Leu Arg Arg Glu Glu Gly Ile
 110 115 120

25 att aaa aat gcg taaatcagca gctcctaagc gtccagtagt tcaggaccct 920
 Ile Lys Asn Ala
 125

30 gtatacaagt ccgagctcgt taccagctc gtaaacaaga tcctcatcgg tggcaagaag 980
 tccaccgcag agcgcacgtc ctacggtgca ctcgagatct gccgtgagaa gaccggcacc 1040
 gatccagtag gaaccctcga gaaggctctc ggcaacgtgc gtccagacct cgaagttcgt 1100

35 tcccgccgtg ttggtggcgc tacctaccag gtgccagtgg atgttcgccc agagcgcgca 1160
 aacaccctcg cactgcgttg gttggttaacc ttcaccgcgc agcgtcgtga gaacaccatg 1220
 atcgagcgtc ttgcaaacga acttctggat gcagccaacg gccttggcgc ttccgtgaag 1280

40 cgtcgcgaag acaccacaaa gatggcagag gccaacgcgc ccttcgctca ctaccgctgg 1340
 tagtactgcc aagacatgaa agcccaatca cttttaagat caacgcctgc cggcgccctt 1400

45 cacatttgaa taagctggca gcctgcgttt cttcaaggcg actgggcttt tagtctcatt 1460
 aatgcagttc accgctgtaa gatagctaaa tagaaacact gtttcggcag tgtgttacta 1520

50 aaaaatccat gtcacttgcc tcgagcgtgc tgcttgaatc gcaagttagt ggcaaaatgt 1580
 aacaagagaa ttatccgtag gtgacaaact ttttaatact tgggtatctg tcatggatac 1640
 cccggttaata aataagtga aatccgtaac caacaagttg gggtaaccact gtggcacaag 1700

55 aagtgcctaa ggatctaaac aagggtccgca acatcggcac catggcgcac atcgatgctg 1760
 gtaagaccac gacca 1775

<210> 8

<211> 127

<212> PRT

<213> Corynebacterium glutamicum

5

<400> 8

Met Pro Thr Ile Gln Gln Leu Val Arg Lys Gly Arg His Asp Lys Ser
1 5 10 15

10 Ala Lys Val Ala Thr Ala Ala Leu Lys Gly Ser Pro Gln Arg Arg Gly
20 25 30

Val Cys Thr Arg Val Tyr Thr Thr Thr Pro Lys Lys Pro Asn Ser Ala
35 40 45

15 Leu Arg Lys Val Ala Arg Val Arg Leu Thr Ser Gly Ile Glu Val Ser
50 55 60

20 Ala Tyr Ile Pro Gly Glu Gly His Asn Leu Gln Glu His Ser Met Val
65 70 75 80

Leu Val Arg Gly Gly Arg Val Lys Asp Leu Pro Gly Val Arg Tyr Lys
85 90 95

25 Ile Val Arg Gly Ala Leu Asp Thr Gln Gly Val Lys Asp Arg Lys Gln
100 105 110

Ala Arg Ser Pro Leu Arg Arg Glu Glu Gly Ile Ile Lys Asn Ala
115 120 125

30

Patentansprüche

1. Isoliertes Polynukleotid aus coryneformen Bakterien, enthaltend eine für das rpoB-Gen kodierende Polynukleotidsequenz, ausgewählt aus der Gruppe
 - 5 a) Polynukleotid, das mindestens zu 70% identisch ist mit einem Polynukleotid, das für ein Polypeptid kodiert, das die Aminosäuresequenz von SEQ ID No. 2 enthält,
 - 10 b) Polynukleotid, das für ein Polypeptid kodiert, das eine Aminosäuresequenz enthält, die zu mindestens 70% identisch ist mit der Aminosäuresequenz von SEQ ID No. 2,
 - c) Polynukleotid, das komplementär ist zu den Polynukleotiden von a) oder b), und
 - 15 d) Polynukleotid, enthaltend mindestens 15 aufeinanderfolgende Nukleotide der Polynukleotidsequenz von a), b) oder c)wobei das Polypeptid bevorzugt die Aktivität der β -Untereinheit der RNA-Polymerase B aufweist.
- 20 2. Polynukleotid gemäß Anspruch 1, wobei das Polynukleotid eine in coryneformen Bakterien replizierbare, bevorzugt rekombinante DNA ist.
3. Polynukleotid gemäß Anspruch 1, wobei das Polynukleotid eine RNA ist.
- 25 4. Polynukleotid gemäß Anspruch 2, enthaltend die Nukleinsäuresequenz wie in SEQ ID No. 1 dargestellt.
5. Replizierbare DNA gemäß Anspruch 2, enthaltend
 - (i) die Nukleotidsequenz, gezeigt in SEQ ID No. 1, oder

- (ii) mindestens eine Sequenz, die der Sequenz
 - (i) innerhalb des Bereichs der Degeneration des genetischen Kodes entspricht, oder
 - (iii) mindestens eine Sequenz, die mit der zur Sequenz
 - (i) oder (ii) komplementären Sequenz hybridisiert, und gegebenenfalls
 - (iv) funktionsneutrale Sinnmutationen in (i).
6. Replizierbare DNA gemäß Anspruch 5, d a d u r c h
g e k e n n z e i c h n e t, daß die Hybridisierung
unter einer Stringenz entsprechend höchstens 2x SSC
durchgeführt wird.
7. Polynukleotidsequenz gemäß Anspruch 1, die für ein
Polypeptid kodiert, das die in SEQ ID No. 2
dargestellte Aminosäuresequenz enthält.
8. Coryneforme Bakterien, in denen das rpoB-Gen verstärkt,
insbesondere überexprimiert wird.
9. Verfahren zur fermentativen Herstellung von L-
Aminosäuren, insbesondere L-Lysin, d a d u r c h
g e k e n n z e i c h n e t, daß man folgende Schritte
durchführt:
- a) Fermentation der die gewünschte L-Aminosäure
produzierenden coryneformen Bakterien, in denen man
zumindest das rpoB-Gen oder dafür kodierende
Nukleotidsequenzen verstärkt, insbesondere
überexprimiert;
 - b) Anreicherung der L-Aminosäure im Medium oder in den
Zellen der Bakterien, und
 - c) Isolieren der L-Aminosäure.
10. Verfahren gemäß Anspruch 9, d a d u r c h
g e k e n n z e i c h n e t, daß man Bakterien

einsetzt, in denen man zusätzlich weitere Gene des Biosyntheseweges der gewünschten L-Aminosäure verstärkt.

- 5 11. Verfahren gemäß Anspruch 9, d a d u r c h
g e k e n n z e i c h n e t, daß man Bakterien
einsetzt, in denen die Stoffwechselwege zumindest
teilweise ausgeschaltet sind, die die Bildung der
gewünschten L-Aminosäure verringern.
- 10 12. Verfahren gemäß Anspruch 9, d a d u r c h
g e k e n n z e i c h n e t, daß man einen mit einem
Plasmidvektor transformierten Stamm einsetzt, und der
Plasmidvektor die für das rpoB-Gen kodierende
Nukleotidsequenz trägt.
- 15 13. Verfahren gemäß Anspruch 9, d a d u r c h
g e k e n n z e i c h n e t, daß man die Expression des
(der) Polynukleotides (e), das (die) für das rpoB-Gen
kodiert (kodieren) verstärkt, insbesondere
überexprimiert.
- 20 14. Verfahren gemäß Anspruch 9, d a d u r c h
g e k e n n z e i c h n e t, daß man die
regulatorischen/katalytischen Eigenschaften des
Polypeptids (Enzymprotein) erhöht, für das das
Polynukleotid rpoB kodiert.
- 25 15. Verfahren gemäß Anspruch 9, d a d u r c h
g e k e n n z e i c h n e t, daß man zur Herstellung
von L-Aminosäuren coryneforme Mikroorganismen
fermentiert, in denen man gleichzeitig eines oder
mehrere der Gene, ausgewählt aus der Gruppe
 - 30 15.1 das für die Dihydrodipicolinat-Synthase
kodierende Gen dapA,
 - 15.2 das für die Glyceraldehyd-3-Phosphat-
Dehydrogenase kodierende Gen gap,

- 15.3 das für die Triosephosphat-Isomerase kodierende Gen tpi,
- 15.4 das für die 3-Phosphoglycerat-Kinase kodierende Gen pgk,
- 5 15.5 das für die Glucose-6-Phosphat-Dehydrogenase kodierende Gen zwf,
- 15.6 das für die Pyruvat-Carboxylase kodierende Gen pyc,
- 10 15.7 das für die Malat-Chinon-Oxidoreduktase kodierende Gen mqo,
- 15.8 das für eine feed-back resistente Aspartatkinase kodierende Gen lysC,
- 15.9 das für den Lysin-Export kodierende Gen lysE,
- 15.10 das für das Zwal-Protein kodierende Gen zwal
- 15 15.11 das für das ribosomale Protein S12 kodierende rpsL-Gen

verstärkt bzw. überexprimiert.

- 20 16. Verfahren gemäß Anspruch 9, d a d u r c h g e k e n n z e i c h n e t, daß man zur Herstellung von L-Aminosäuren coryneforme Mikroorganismen fermentiert, in denen man gleichzeitig eines oder mehrere der Gene, ausgewählt aus der Gruppe

- 25 16.1 das für die Phosphoenolpyruvat-Carboxykinase kodierende Gen pck,
- 16.2 das für die Glucose-6-Phosphat Isomerase kodierende Gen pgi,
- 16.3 das für die Pyruvat-Oxidase kodierende Gen poxB

16.4 das für das Zwa2-Protein kodierende Gen zwa2
abschwächt.

17. Coryneforme Bakterien, die einen Vektor enthalten, der ein Polynukleotid gemäß Anspruch 1 trägt.
- 5 18. Verfahren gemäß einem oder mehreren der vorhergehenden Ansprüche, d a d u r c h g e k e n n z e i c h n e t, daß man Mikroorganismen der Art Corynebacterium glutamicum einsetzt.
- 10 19. Verfahren zum Auffinden von RNA, cDNA und DNA, um Nukleinsäuren, beziehungsweise Polynukleotide oder Gene zu isolieren, die für die β -Untereinheit der RNA-Polymerase B kodieren oder eine hohe Ähnlichkeit mit der Sequenz des rpoB-Gens aufweisen, d a d u r c h g e k e n n z e i c h n e t, daß man das Polynukleotid, 15 enthaltend die Polynukleotidsequenzen gemäß den Ansprüchen 1, 2, 3 oder 4, als Hybridisierungs sonden einsetzt.
- 20 20. Verfahren gemäß Anspruch 18, d a d u r c h g e k e n n z e i c h n e t, daß man arrays, micro arrays oder DNA-chips einsetzt.
- 25 21. Aus coryneformen Bakterien stammende DNA, kodierend für β -Untereinheiten der RNA-Polymerase B, wobei die zugehörigen Aminosäuresequenzen zwischen den Positionen 1 bis 10 in der SEQ ID No. 2 durch Aminosäureaustausch verändert sind.
- 30 22. DNA gemäß Anspruch 21 d a d u r c h g e k e n n z e i c h n e t, daß diese für β -Untereinheiten der RNA-Polymerase B kodieren, wobei die zugehörigen Aminosäuresequenzen an Position 5 in der SEQ ID No. 2 L-Leucin, L-Isoleucin oder L-Valin enthalten.

23. Aus coryneformen Bakterien stammende DNA, kodierend für β -Untereinheiten der RNA-Polymerase B, wobei die zugehörigen Aminosäuresequenzen zwischen den Positionen 190 bis 200 in der SEQ ID No. 2 durch
5 Aminosäureaustausch verändert sind.
24. DNA gemäß Anspruch 23 d a d u r c h
g e k e n n z e i c h n e t, daß diese für β -Untereinheiten der RNA-Polymerase B kodieren, wobei die zugehörigen Aminosäuresequenzen an Position 196 in der
10 SEQ ID No. 2 L-Phenylalanin oder L-Tyrosin enthalten.
25. Aus coryneformen Bakterien stammende DNA, kodierend für β -Untereinheiten der RNA-Polymerase B, wobei die zugehörigen Aminosäuresequenzen zwischen den Positionen 420 bis 450 in der SEQ ID No. 2 durch
15 Aminosäureaustausch verändert sind.
26. DNA gemäß Anspruch 25 d a d u r c h
g e k e n n z e i c h n e t, daß diese für die β -Untereinheiten der RNA-Polymerase B kodieren, wobei die zugehörigen Aminosäuresequenzen an den Positionen 439
20 in der SEQ ID No. 2 jede andere proteinogene Aminosäure ausgenommen L-Histidin enthalten.
27. DNA gemäß Anspruch 25 d a d u r c h
g e k e n n z e i c h n e t, daß diese für die β -Untereinheiten der RNA-Polymerase B kodieren, wobei die zugehörigen Aminosäuresequenzen an Position 424 in der
25 SEQ ID No. 2 L-Prolin oder L-Arginin enthalten.
28. DNA gemäß Anspruch 25 d a d u r c h
g e k e n n z e i c h n e t, daß diese für die β -Untereinheiten der RNA-Polymerase B kodieren, wobei die zugehörigen Aminosäuresequenzen an Position 425 in der
30 SEQ ID No. L-Threonin oder L-Alanin enthalten.
29. DNA gemäß Anspruch 25 d a d u r c h
g e k e n n z e i c h n e t, daß diese für die β -

Untereinheiten der RNA-Polymerase B kodieren, wobei die zugehörigen Aminosäuresequenzen an Position 426 in der SEQ ID No. 2 L-Leucin oder L-Lysin enthalten.

- 5 30. DNA gemäß Anspruch 25 d a d u r c h
g e k e n n z e i c h n e t, daß diese für die β -
Untereinheiten der RNA-Polymerase B kodieren, wobei die
zugehörigen Aminosäuresequenzen an Position 429 in der
SEQ ID No. L-Isoleucin, L-Valin oder L-Leucin
enthalten.
- 10 31. DNA gemäß Anspruch 25 d a d u r c h
g e k e n n z e i c h n e t, daß diese für die β -
Untereinheiten der RNA-Polymerase B kodieren, wobei die
zugehörigen Aminosäuresequenzen an Position 444 in der
SEQ ID No. 2 L-Leucin, L- Tyrosin oder L-Tryptophan
15 enthalten.
32. DNA gemäß Anspruch 25 d a d u r c h
g e k e n n z e i c h n e t, daß diese für die β -
Untereinheiten der RNA-Polymerase B kodieren, wobei die
zugehörigen Aminosäuresequenzen an Position 446 in der
20 SEQ ID No. 2 L-Prolin oder L-Isoleucin enthalten.
- 25 33. Aus coryneformen Bakterien stammende DNA, kodierend für
 β -Untereinheiten der RNA-Polymerase B, wobei die
zugehörigen Aminosäuresequenzen an einer oder mehreren
Positionen ausgewählt aus der Gruppe a) Position 1 bis
10, b) Position 190 bis 200 und c) Position 420 bis 450
in der SEQ ID No. 2 gleichzeitig durch
Aminosäureaustausch verändert sind.
- 30 34. DNA gemäß Anspruch 33 d a d u r c h
g e k e n n z e i c h n e t, daß diese für die β -
Untereinheiten der RNA-Polymerase B kodieren, wobei die
zugehörigen Aminosäuresequenzen in der SEQ ID No. 2 an
einer oder mehreren Positionen ausgewählt aus der
Gruppe a) Position 5 L-Leucin, b) Position 196 L-

Phenylalanin, c) Position 429 L-Valin, und d) Position 439 L-Tyrosin, enthalten.

35. DNA gemäß Anspruch 34 d a d u r c h
g e k e n n z e i c h n e t, daß diese für die β -
Untereinheit der RNA-Polymerase B kodiert, wobei die
zugehörige Aminosäuresequenz an Position 5 L-Leucin, an
Position 196 L-Phenylalanin, und an Position 429 L-
Valin, enthält, dargestellt in SEQ ID No. 4.
36. DNA gemäß Anspruch 34 d a d u r c h
g e k e n n z e i c h n e t, daß diese für die β -
Untereinheit der RNA-Polymerase B kodiert, wobei die
zugehörigen Aminosäuresequenz an der Position 439 L-
Tyrosin enthält, dargestellt in SEQ ID No. 6.
37. Aus coryneformen Bakterien stammende DNA, kodierend für
die β -Untereinheit der RNA-Polymerase B, wobei die
Basensequenz der DNA an der Position 715 Thymin
enthält, an der Position 1288 Thymin enthält, und an
der Position 1987 Thymin enthält, dargestellt in SEQ ID
No. 3.
38. Aus coryneformen Bakterien stammende DNA, kodierend für
die β -Untereinheit der RNA-Polymerase B, wobei die
Basensequenz der DNA an der Position 2016 Thymin
enthält, dargestellt in SEQ ID No. 5.

Zusammenfassung

Die Erfindung betrifft ein isoliertes Polynukleotid, enthaltend eine Polynukleotidsequenz, ausgewählt aus der Gruppe

- 5 a) Polynukleotid, das mindestens zu 70% identisch ist mit einem Polynukleotid, das für ein Polypeptid kodiert, das die Aminosäuresequenz von SEQ ID No. 2 enthält,
- b) Polynukleotid, das für ein Polypeptid kodiert, das eine Aminosäuresequenz enthält, die zu mindestens 70%
10 identisch ist mit der Aminosäuresequenz von SEQ ID No. 2,
- c) Polynukleotid, das komplementär ist zu den Polynukleotiden von a) oder b), und
- d) Polynukleotid, enthaltend mindestens 15
15 aufeinanderfolgende Nukleotide der Polynukleotidsequenz von a), b) oder c),

und ein Verfahren zur fermentativen Herstellung von L-Aminosäuren unter Verwendung von coryneformen Bakterien, in denen zumindest das rpoB-Gen verstärkt vorliegt, und die
20 Verwendung von Polynukleotiden, die die erfindungsgemäßen Sequenzen enthalten, als Hybridisierungs sonden.

TRANSLATOR'S DECLARATION

1033 U.S. PTO
09/887052
06/25/01

I, Judith Atkinson, BA(Hons.), MITI., translator to Messrs. Taylor & Meyer of 20 Kingsmead Road, London, SW2 3JD, Great Britain, verify that I know well both the German and the English language, that I have prepared the attached English translation of pages of a German Patent application in the German language with the title:

Neue für das rpoB-Gen kodierende Nukleotidsequenzen

identified by the code number 000781 BT at the upper left of each page and corresponding to client/matter number of the law firm of

and that the attached English translation of this document is a true and correct translation of the document attached thereto to the best of my knowledge and belief.

I further declare that all statements made of my own knowledge are true and that all statements made on information and belief are believed to be true, and further that these statements are made with the knowledge that wilful false statements and the like are punishable by fine or imprisonment, or both, under 18 USC 1001, and that such false statements may jeopardize the validity of this document.

Date: 13th March 2001

By: J. M. Atkinson.



Novel nucleotide sequences coding for the rpoB gene

The invention provides nucleotide sequences from coryneform bacteria coding for the rpoB gene, and a process for the production of amino acids by fermentation using bacteria in
5 which the rpoB gene is enhanced.

Prior art

L-amino acids, especially L-lysine, are used in human medicine and in the pharmaceuticals industry, in the foodstuffs industry and, very especially, in the feeding of
10 animals.

It is known that amino acids are produced by fermentation of strains of coryneform bacteria, especially *Corynebacterium glutamicum*. Because of their great importance, attempts are continuously being made to improve
15 the production processes. Improvements to the processes may concern measures relating to the fermentation, such as, for example, stirring and oxygen supply, or the composition of the nutrient media, such as, for example, the sugar concentration during the fermentation, or working up to the
20 product form by, for example, ion-exchange chromatography, or the intrinsic performance properties of the microorganism itself.

In order to improve the performance properties of such microorganisms, methods of mutagenesis, selection and
25 mutant selection are employed. Such methods yield strains which are resistant to antimetabolites or are auxotrophic for metabolites that are important in terms of regulation, and which produce amino acids.

For a number of years, methods of recombinant DNA
30 technology have also been used for improving the strain of L-amino acid-producing strains of *Corynebacterium*, by

amplifying individual amino acid biosynthesis genes and studying the effect on amino acid production.

Object of the invention

The inventors have set themselves the object of providing
5 novel measures for the improved production of amino acids by fermentation.

Description of the invention

Where L-amino acids or amino acids are mentioned hereinbelow, they are to be understood as meaning one or
10 more amino acids, including their salts, selected from the group L-asparagine, L-threonine, L-serine, L-glutamate, L-glycine, L-alanine, L-cysteine, L-valine, L-methionine, L-isoleucine, L-leucine, L-tyrosine, L-phenylalanine, L-histidine, L-lysine, L-tryptophan and L-arginine. L-lysine
15 is especially preferred.

Where L-lysine or lysine is mentioned hereinbelow, it is to be understood as meaning not only the bases but also the salts, such as, for example, lysine monohydrochloride or lysine sulfate.

20 The invention provides an isolated polynucleotide from coryneform bacteria, containing a polynucleotide sequence coding for the rpoB gene, selected from the group

a) polynucleotide that is at least 70% identical with a polynucleotide that codes for a polypeptide containing
25 the amino acid sequence of SEQ ID No. 2,

b) polynucleotide that codes for a polypeptide containing an amino acid sequence that is at least 70% identical with the amino acid sequence of SEQ ID No. 2,

c) polynucleotide that is complementary to the
30 polynucleotides of a) or b), and

- d) polynucleotide containing at least 15 consecutive nucleotides of the polynucleotide sequence of a), b) or c),

the polypeptide preferably exhibiting the activity of the β -subunit of RNA polymerase B.

The invention also provides the above-mentioned polynucleotide, it preferably being a replicatable DNA containing:

- (i) the nucleotide sequence shown in SEQ ID No. 1, or
- 10 (ii) at least one sequence that corresponds to sequence (i) within the region of the degeneracy of the genetic code, or
- (iii) at least one sequence that hybridizes with the sequence that is complementary to sequence (i) or (ii), and optionally
- 15 (iv) sense mutations in (i) which are neutral in terms of function and which do not change the activity of the protein/polypeptide.

Finally, the invention also provides polynucleotides selected from the group

- a) polynucleotides containing at least 15 consecutive nucleotides selected from the nucleotide sequence of SEQ ID No. 1 between positions 1 and 701
- 25 b) polynucleotides containing at least 15 consecutive nucleotides selected from the nucleotide sequence of SEQ ID No. 1 between positions 702 and 4199
- c) polynucleotides containing at least 15 consecutive nucleotides selected from the nucleotide sequence of SEQ ID No. 1 between positions 4200 and 5099.

The invention also provides

a replicatable polynucleotide, especially DNA, containing the nucleotide sequence as shown in SEQ ID No. 1;

5 a polynucleotide that codes for a polypeptide containing the amino acid sequence as shown in SEQ ID No. 2;

a vector containing the polynucleotide of the invention, especially a shuttle vector or a plasmid vector, and

coryneform bacteria which contain the vector or in which the rpoB gene has been enhanced.

10 The invention also provides polynucleotides consisting substantially of a polynucleotide sequence, which are obtainable by screening, by means of hybridization, a corresponding gene library of a coryneform bacteria that contains the complete gene or parts thereof, using a probe
15 containing the sequence of the polynucleotide of the invention according to SEQ ID No. 1 or a fragment thereof, and isolating the mentioned polynucleotide sequence.

Polynucleotides that contain the sequences of the invention are suitable as hybridization probes for RNA, cDNA and DNA,
20 in order to isolate in their complete length nucleic acids or polynucleotides or genes that code for the β -subunit of RNA polymerase B, or in order to isolate nucleic acids or polynucleotides or genes that are very similar to the sequence of the rpoB gene. They are likewise suitable for
25 incorporation into so-called "arrays", "micro arrays" or "DNA chips" in order to detect and determine the corresponding polynucleotides.

Polynucleotides that contain the sequences of the invention are also suitable as primers, with the aid of which it is
30 possible, by means of the polymerase chain reaction (PCR), to produce DNA of genes that code for the β -subunit of RNA polymerase B.

Such oligonucleotides acting as probes or primers contain at least 25, 26, 27, 28, 29 or 30, preferably at least 20, 21, 22, 23 or 24, very especially preferably at least 15, 16, 17, 18 or 19, consecutive nucleotides. Also suitable
5 are oligonucleotides having a length of at least 31, 32, 33, 34, 35, 36, 37, 38, 39 or 40 or of at least 41, 42, 43, 44, 45, 46, 47, 48, 49 or 50 nucleotides. Oligonucleotides having a length of at least 100, 150, 200, 250 or 300 nucleotides may also be suitable.

10 "Isolated" means removed from its natural environment.

"Polynucleotide" generally refers to polyribonucleotides and polydeoxyribonucleotides, it being possible for the RNA or DNA to be unmodified or modified.

The polynucleotides of the invention include a
15 polynucleotide according to SEQ ID No. 1 or a fragment prepared therefrom, and also polynucleotides that are at least especially from 70% to 80%, preferably at least from 81% to 85%, especially preferably at least from 86% to 90%, and very especially preferably at least 91%, 93%, 95%, 97%
20 or 99%, identical with the polynucleotide according to SEQ ID No. 1, or with a fragment prepared therefrom.

"Polypeptides" are to be understood as being peptides or proteins that contain two or more amino acids bonded via peptide bonds.

25 The polypeptides of the invention include a polypeptide according to SEQ ID No. 2, especially those having the biological activity of the β -subunit of RNA polymerase B, and also those that are at least from 70% to 80%, preferably at least from 81% to 85%, especially preferably
30 at least from 86% to 90%, and very especially preferably at least 91%, 93%, 95%, 97% or 99%, identical with the polypeptide according to SEQ ID No. 2 and exhibit the mentioned activity.

The invention also provides a process for the production, by fermentation, of amino acids selected from the group L-asparagine, L-threonine, L-serine, L-glutamate, L-glycine, L-alanine, L-cysteine, L-valine, L-methionine, L-

- 5 isoleucine, L-leucine, L-tyrosine, L-phenylalanine, L-histidine, L-lysine, L-tryptophan and L-arginine, using coryneform bacteria which, in particular, already produce amino acids and in which the nucleotide sequences coding for the rpoB gene are enhanced, especially overexpressed.
- 10 The term "enhancement" in this connection describes the increasing of the intracellular activity of one or more enzymes or proteins in a microorganism that are coded for by the corresponding DNA, by, for example, increasing the number of copies of the gene or genes, using a strong
- 15 promoter or using a gene or allele that codes for a corresponding enzyme or protein having a high level of activity, and optionally by combining those measures.

- The microorganisms provided by the present invention can produce L-amino acids from glucose, saccharose, lactose,
- 20 fructose, maltose, molasses, starch, cellulose or from glycerol and ethanol. They may be representatives of coryneform bacteria, especially of the genus *Corynebacterium*. In the case of the genus *Corynebacterium*, special mention may be made of the species *Corynebacterium glutamicum*, which is known to those skilled in the art for
- 25 its ability to produce L-amino acids.

Suitable strains of the genus *Corynebacterium*, especially of the species *Corynebacterium glutamicum* (*C. glutamicum*), are especially the known wild-type strains

- 30 *Corynebacterium glutamicum* ATCC13032
Corynebacterium acetoglutamicum ATCC15806
Corynebacterium acetoacidophilum ATCC13870
Corynebacterium thermoaminogenes FERM BP-1539
Corynebacterium melassecola ATCC17965

Brevibacterium flavum ATCC14067
Brevibacterium lactofermentum ATCC13869 and
Brevibacterium divaricatum ATCC14020

and L-amino acid-producing mutants or strains prepared
5 therefrom, such as, for example, the L-lysine-producing
strains

Corynebacterium glutamicum FERM-P 1709
Brevibacterium flavum FERM-P 1708
Brevibacterium lactofermentum FERM-P 1712
10 Corynebacterium glutamicum FERM-P 6463
Corynebacterium glutamicum FERM-P 6464
Corynebacterium glutamicum DM58-1
Corynebacterium glutamicum DG52-5
Corynebacterium glutamicum DSM5714 and
15 Corynebacterium glutamicum DSM12866.

The new rpoB gene of C. glutamicum coding for the β -subunit
of RNA polymerase B has been isolated.

In order to isolate the rpoB gene or other genes from C.
glutamicum, a gene library of that microorganism in
20 Escherichia coli (E. coli) is first prepared. The
preparation of gene libraries is written down in generally
known textbooks and handbooks. There may be mentioned as an
example the textbook of Winnacker: Gene und Klone, Eine
Einführung in die Gentechnologie (Verlag Chemie, Weinheim,
25 Germany, 1990) or the handbook of Sambrook et al.:
Molecular Cloning, A Laboratory Manual (Cold Spring Harbor
Laboratory Press, 1989). A very well known gene library is
that of the E. coli K-12 strain W3110, which has been
prepared by Kohara et al. (Cell 50, 495-508 (1987)) in λ -
30 vectors. Bathe et al. (Molecular and General Genetics,
252:255-265, 1996) describe a gene library of C. glutamicum
ATCC13032, which has been prepared with the aid of the
cosmid vector SuperCos I (Wahl et al., 1987, Proceedings of
the National Academy of Sciences USA, 84:2160-2164) in the

E. coli K-12 strain NM554 (Raleigh et al., 1988, Nucleic Acids Research 16:1563-1575).

Börmann et al. (Molecular Microbiology 6(3), 317-326) (sic) (1992)) in turn describe a gene library of C. glutamicum
5 ATCC13032 using the cosmid pHc79 (Hohn and Collins, Gene 11, 291-298 (1980)).

For the preparation of a gene library of C. glutamicum in E. coli it is also possible to use plasmids such as pBR322 (Bolivar, Life Sciences, 25, 807-818 (1979)) or pUC9
10 (Vieira et al., 1982, Gene, 19:259-268). Suitable hosts are especially those E. coli strains that are restriction- and recombination-defective. An example thereof is the strain DH5 α mc^r, which has been described by Grant et al. (Proceedings of the National Academy of Sciences USA, 87
15 (1990) 4645-4649). The long DNA fragments cloned with the aid of cosmids can then in turn be subcloned into customary vectors suitable for sequencing and then sequenced, as is described, for example, in Sanger et al. (Proceedings of the National Academy of Sciences of the United States of
20 America, 74:5463-5467, 1977).

The resulting DNA sequences can then be studied using known algorithms or sequence-analysis programs, such as, for example, that of Staden (Nucleic Acids Research 14, 217-232 (1986)), that of Marck (Nucleic Acids Research 16,
25 1829-1836 (1988)) or the GCG program of Butler (Methods of Biochemical Analysis 39, 74-97 (1998)).

The novel DNA sequence of C. glutamicum coding for the rpoB gene has been found and, as SEQ ID No. 1, forms part of the present invention. Furthermore, the amino acid sequence of
30 the corresponding protein has been derived from the present DNA sequence using the methods described above. The resulting amino acid sequence of the rpoB gene product is shown in SEQ ID No. 2. It is known that enzymes belonging to the host are able to cleave the N-terminal amino acid

methionine or formylmethionine of the protein that is formed.

Coding DNA sequences that result from SEQ ID No. 1 by the degeneracy of the genetic code also form part of the invention. Likewise, DNA sequences that hybridize with SEQ ID No. 1 or parts of SEQ ID No. 1 form part of the invention. Furthermore, to those skilled in the art, conservative amino acid substitutions, such as, for example, the substitution of glycine with alanine or of aspartic acid with glutamic acid, in proteins are known as sense mutations, which do not lead to any fundamental change in the activity of the protein, that is to say are neutral in terms of function. Such mutations are known *inter alia* also as neutral substitutions. It is also known that changes at the N- and/or C-terminus of a protein do not substantially impair its function or may even stabilise it. The person skilled in the art will find relevant information *inter alia* in Ben-Bassat *et al.* (Journal of Bacteriology 169:751-757 (1987)), in O'Regan *et al.* (Gene 77:237-251 (1989)), in Sahin-Toth *et al.* (Protein Sciences 3:240-247 (1994)), in Hochuli *et al.* (Bio/Technology 6:1321-1325 (1988)) and in known textbooks of genetics and molecular biology. Amino acid sequences that result in a corresponding manner from SEQ ID No. 2 likewise form part of the invention.

Similarly, DNA sequences that hybridize with SEQ ID No. 1 or parts of SEQ ID No. 1 form part of the invention. Finally, DNA sequences that are produced by the polymerase chain reaction (PCR) using primers that result from SEQ ID No. 1 form part of the invention. Such oligonucleotides typically have a length of at least 15 nucleotides.

The person skilled in the art will find instructions on the identification of DNA sequences by means of hybridization *inter alia* in the handbook "The DIG System Users Guide for Filter Hybridization" from Boehringer Mannheim GmbH

(Mannheim, Germany, 1993) and in Liebl et al.

(International Journal of Systematic Bacteriology (1991)

41: 255-260). The hybridization takes place under stringent conditions, that is to say there are formed only hybrids in

5 which the probe and the target sequence, i.e. the polynucleotides treated with the probe, are at least 70% identical. It is known that the stringency of the hybridization, including the washing steps, is influenced or determined by varying the buffer composition, the
10 temperature and the salt concentration. The hybridization reaction is preferably carried out with relatively low stringency as compared with the washing steps (Hybaid Hybridisation Guide, Hybaid Limited, Teddington, UK, 1996).

There may be used for the hybridization reaction, for
15 example, a 5x SSC buffer at a temperature of approximately from 50°C to 68°C. In that case, probes may also hybridize with polynucleotides that are less than 70% identical with the sequence of the probe. Such hybrids are less stable and are removed by washing under stringent conditions. That may
20 be achieved, for example, by lowering the salt concentration to 2x SSC and optionally subsequently to 0.5x SSC (The DIG System User's Guide for Filter Hybridisation, Boehringer Mannheim, Mannheim, Germany, 1995), a temperature of approximately from 50°C to 68°C being set.
25 It is optionally possible to lower the salt concentration down to 0.1x SSC. By raising the hybridization temperature stepwise from 50°C to 68°C in steps of approximately from 1 to 2°C, it is possible to isolate polynucleotide fragments that are, for example, at least 70% or at least 80% or at
30 least from 90% to 95% identical with the sequence of the probe used. Further instructions for hybridization are commercially available in the form of so-called kits (e.g. DIG Easy Hyb from Roche Diagnostics GmbH, Mannheim, Germany, Catalog No. 1603558).

The person skilled in the art will find instructions on the amplification of DNA sequences with the aid of the polymerase chain reaction (PCR) *inter alia* in the handbook of Gait: Oligonukleotide synthesis: A Practical Approach
5 (IRL Press, Oxford, UK, 1984) and in Newton and Graham: PCR (Spektrum Akademischer Verlag, Heidelberg, Germany, 1994).

It has been found that coryneform bacteria produce amino acids in an improved manner after enhancement of the *rpoB* gene.

10 In order to achieve overexpression, the number of copies of the corresponding genes can be increased, or the promoter and regulation region or the ribosome binding site, which is located upstream of the structural gene, can be mutated. Expression cassettes inserted upstream of the structural
15 gene have a similar effect. By means of inducible promoters it is additionally possible to increase the expression in the course of the production of amino acids by fermentation. Expression is also improved by measures to prolong the life of the m-RNA. Furthermore, the enzyme
20 activity is also enhanced by preventing degradation of the enzyme protein. The genes or gene constructs may either be present in plasmids with different numbers of copies or be integrated and amplified in the chromosome. Alternatively, overexpression of the genes in question may also be
25 achieved by changing the composition of the medium and the manner in which culturing is carried out.

The person skilled in the art will find instructions thereon in Martin et al. (Bio/Technology 5, 137-146 (1987)), in Guerrero et al. (Gene 138, 35-41 (1994)),
30 Tsuchiya and Morinaga (Bio/Technology 6, 428-430 (1988)), in Eikmanns et al. (Gene 102, 93-98 (1991)), in European patent specification 0 472 869, in US patent 4,601,893, in Schwarzer and Pühler (Bio/Technology 9, 84-87 (1991)), in Reinscheid et al. (Applied and Environmental Microbiology
35 60, 126-132 (1994)), in LaBarre et al. (Journal of

Bacteriology 175, 1001-1007 (1993)), in patent application WO 96/15246, in Malumbres et al. (Gene 134, 15-24 (1993)), in Japanese Offenlegungsschrift JP-A-10-229891, in Jensen and Hammer (Biotechnology and Bioengineering 58, 191-195
5 (1998)), in Makrides (Microbiological Reviews 60:512-538 (1996)) and in known textbooks of genetics and molecular biology.

For the purposes of enhancement, the rpoB gene of the invention was overexpressed, for example, with the aid of
10 episomal plasmids. Suitable plasmids are those which are replicated in coryneform bacteria. Many known plasmid vectors, such as, for example, pZ1 (Menkel et al., Applied and Environmental Microbiology (1989) 64: 549-554), pEKEx1 (Eikmanns et al., Gene 102:93-98 (1991)) or pHS2-1 (Sonnen
15 et al., Gene 107:69-74 (1991)), are based on the cryptic plasmids pHM1519, pBL1 or pGA1. Other plasmid vectors, such as, for example, those which are based on pCG4 (US-A 4,489,160) or pNG2 (Serwold-Davis et al., FEMS Microbiology Letters 66, 119-124 (1990)) or pAG1 (US-A 5,158,891), may
20 likewise be used.

Also suitable are those plasmid vectors with the aid of which the process of gene amplification by integration into the chromosome can be applied, as has been described, for example, by Reinscheid et al. (Applied and Environmental
25 Microbiology 60, 126-132 (1994)) for the duplication or amplification of the hom-thrB operon. In that method, the complete gene is cloned into a plasmid vector that is able to replicate in a host (typically E. coli), but not in C. glutamicum. Suitable vectors are, for example, pSUP301
30 (Simon et al., Bio/Technology 1, 784-791 (1983)), pK18mob or pK19mob (Schäfer et al., Gene 145, 69-73 (1994)), pGEM-T (Promega corporation, Madison, WI, USA), pCR2.1-TOPO (Shuman (1994). Journal of Biological Chemistry 269:32678-32684; US-A 5,487,993), pCR®Blunt (Invitrogen,
35 Groningen, Netherlands; Bernard et al., Journal of

Molecular Biology, 234: 534-541 (1993)), pEM1 (Schrumpf et al., 1991, Journal of Bacteriology 173:4510-4516) or pBGS8 (Spratt et al., 1986, Gene 41: 337-342). The plasmid vector containing the gene to be amplified is then transferred to
5 the desired strain of *C. glutamicum* by conjugation or transformation. The method of conjugation is described, for example, in Schäfer et al. (Applied and Environmental Microbiology 60, 756-759 (1994)). Methods of transformation are described, for example, in Thierbach et al. (Applied
10 Microbiology and Biotechnology 29, 356-362 (1988)), Dunican and Shivnan (Bio/Technology 7, 1067-1070 (1989)) and Tauch et al. (FEMS Microbiological Letters 123, 343-347 (1994)). After homologous recombination by means of a "cross-over" occurrence, the resulting strain contains at least two
15 copies of the gene in question.

It has also been found that the substitution of amino acids, especially in the sections between position 1 to 10, 190 to 200 and 420 to 450 in the amino acid sequence of the β -subunit of RNA polymerase B shown in SEQ ID No. 2,
20 improves the lysine production of coryneform bacteria.

It has also been found that the substitution of amino acids at one or more positions selected from the group a) position 1 to 10, b) position 190 to 200 and c) position 420 to 450 in SEQ ID No. 2 may take place simultaneously.

25 In the region between position 1 to 10, preference is given to the substitution of L-proline at position 5 by L-leucine, L-isoleucine or L-valine.

In the region between position 190 to 200, preference is given to the substitution of L-serine at position 196 by L-
30 phenylalanine or L-tyrosine.

In the region between 420 to 450, the following substitutions are preferred: substitution of L-leucine at position 424 by L-proline or L-arginine, substitution of

- L-serine at position 425 by L-threonine or L-alanine, substitution of L-glutamine at position 426 by L-leucine or L-lysine, substitution of L-aspartic acid at position 429 by L-isoleucine, L-valine or L-leucine, substitution of L-histidine at position 439 by any proteinogenic amino acid with the exception of L-histidine, is (sic) the substitution of L-serine at position 444 by L-leucine, L-tyrosine or L-tryptophan, and substitution of L-leucine at position 446 by L-proline or L-isoleucine.
- 10 Very special preference is given to one or more amino acid substitutions selected from the group: L-proline at position 5 by L-leucine, L-serine at position 196 by L-phenylalanine, L-aspartate at position 429 by L-valine, and L-histidine at position 439 by L-tyrosine.
- 15 SEQ ID No. 3 shows the base sequence of the allele rpoB-1547 contained in strain DM1547. The rpoB-1547 allele codes for a protein the amino acid sequence of which is shown in SEQ ID No. 4. The protein contains L-leucine at position 5, L-phenylalanine at position 196 and L-valine at position
- 20 429. The DNA sequence of the rpoB-1547 allele (SEQ ID No. 3) contains the following base substitutions as compared with the rpoB wild-type gene (SEQ ID No. 1): thymine at position 715 instead of cytosine, thymine at position 1288 instead of cytosine, and thymine at position
- 25 1987 instead of adenine.
- SEQ ID No. 5 shows the base sequence of the allele rpoB-1546 contained in strain DM1546. The rpoB-1546 allele codes for a protein the amino acid sequence of which is shown in SEQ ID No. 6. The protein contains L-tyrosine at position
- 30 439. The DNA sequence of the rpoB-1546 allele (SEQ ID No. 5) contains the following base substitutions as compared with the rpoB wild-type gene (SEQ ID No. 1): thymine at position 2016 instead of cytosine.

- There may be employed for the mutagenesis conventional methods of mutagenesis using mutagenic substances such as, for example, N-methyl-N'-nitro-N-nitrosoguanidine or ultraviolet light. There may also be used for the
- 5 mutagenesis *in vitro* methods such as, for example, treatment with hydroxylamine (Miller, J. H.: A Short Course in Bacterial Genetics. A Laboratory Manual and Handbook for *Escherichia coli* and Related Bacteria, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, 1992) or mutagenic
- 10 oligonucleotides (T. A. Brown: Gentechnologie für Einsteiger, Spektrum Akademischer Verlag, Heidelberg, 1993) or the polymerase chain reaction (PCR), as is described in the handbook of Newton and Graham (PCR, Spektrum Akademischer Verlag, Heidelberg, 1994).
- 15 In addition, it may be advantageous for the production of L-amino acids to enhance, especially to overexpress, in addition to the *rpoB* gene, one or more enzymes of the biosynthesis pathway in question, of glycolysis, of the anaplerotic pathway, of the citric acid cycle, of the
- 20 pentose phosphate cycle, of amino acid export, and, optionally, regulatory proteins.

Accordingly, for the production of L-lysine, in addition to enhancing the *rpoB* gene, one or more genes selected from the group

- 25 • the gene *dapA* coding for dihydrodipicolinate synthase (EP-B 0 197 335),
- the gene *gap* coding for glyceraldehyde 3-phosphate dehydrogenase (Eikmanns (1992), Journal of Bacteriology 174:6076-6086),
- 30 • the gene *tpi* coding for triose phosphate isomerase (Eikmanns (1992), Journal of Bacteriology 174:6076-6086),
- the gene *pgk* coding for 3-phosphoglycerate kinase (Eikmanns (1992), Journal of Bacteriology 174:6076-6086),

- the gene zwf coding for glucose-6-phosphate dehydrogenase (JP-A-09224661),
- the gene pyc coding for pyruvate carboxylase (DE-A-198 31 609),
- 5 ◦ the gene mgo coding for malate quinone oxidoreductase (Molenaar et al., European Journal of Biochemistry 254, 395-403 (1998)),
- the gene lysC coding for a feed-back resistant aspartate kinase (Kalinowski et al., Molecular Microbiologie 5(5),
10 1197-1204 (1991)),
- the gene lysE coding for lysine export (DE-A-195 48 222),
- the gene zwal coding for the Zwal protein (DE: 19959328.0, DSM 13115), and
- the rpsL gene coding for ribosomal protein S12 and shown
15 in SEQ ID No. 7 and 8

may be enhanced, especially overexpressed.

The term "attenuation" in this connection describes the diminution or exclusion of the intracellular activity of one or more enzymes (proteins) in a microorganism that are
20 coded for by the corresponding DNA, by, for example, using a weak promoter or using a gene or allele that codes for a corresponding enzyme having low activity, or by inactivating the corresponding gene or enzyme (protein), and optionally by combining those measures.

25 Furthermore, it may be advantageous for the production of L-amino acids, in addition to enhancing the rpoB gene, to attenuate, especially to diminish the expression of, one or more genes selected from the group

- the gene pck coding for phosphoenol pyruvate carboxykinase (DE 199 50 409.1; DSM 13047),
- the gene pgi coding for glucose-6-phosphate isomerase (US 09/396,478; DSM 12969),
- 5 ◦ the gene poxB coding for pyruvate oxidase (DE: 1995 1975.7; DSM 13114),
- the gene zwa2 coding for the Zwa2 protein (DE: 19959327.2, DSM 13113).

10 It may also be advantageous for the production of amino acids, in addition to enhancing the rpoB gene, to exclude undesired secondary reactions (Nakayama: "Breeding of Amino Acid Producing Micro-organisms", in: Overproduction of Microbial Products, Krumphanzl, Sikyta, Vanek (eds.), Academic Press, London, UK, 1982).

15 The microorganisms produced according to the invention also form part of the invention and can be cultivated, for the purposes of the production of amino acids, continuously or discontinuously in the batch, fed batch or repeated fed batch process. A summary of known cultivation methods is
20 described in the textbook of Chmiel (Bioprozeßtechnik 1. Einführung in die Bioverfahrenstechnik (Gustav Fischer Verlag, Stuttgart, 1991)) or in the textbook of Storhas (Bioreaktoren und periphere Einrichtungen (Vieweg Verlag, Braunschweig/Wiesbaden, 1994)).

25 The culture medium to be used must meet the requirements of the strains in question in a suitable manner. Descriptions of culture media for various microorganisms are to be found in the handbook "Manual of Methods for General Bacteriology" of the American Society for Bacteriology
30 (Washington D.C., USA, 1981).

There may be used as the carbon source sugars and carbohydrates, such as, for example, glucose, saccharose,

lactose, fructose, maltose, molasses, starch and cellulose, oils and fats, such as, for example, soybean oil, sunflower oil, groundnut oil and coconut oil, fatty acids, such as, for example, palmitic acid, stearic acid and linoleic acid, 5 alcohols, such as, for example, glycerol and ethanol, and organic acids, such as, for example, acetic acid. Those substances may be used individually or in the form of a mixture.

There may be used as the nitrogen source organic nitrogen- 10 containing compounds, such as peptones, yeast extract, meat extract, malt extract, corn steep liquor, soybean flour and urea, or inorganic compounds, such as ammonium sulfate, ammonium chloride, ammonium phosphate, ammonium carbonate and ammonium nitrate. The nitrogen sources may be used 15 individually or in the form of a mixture.

There may be used as the phosphorus source phosphoric acid, potassium dihydrogen phosphate or dipotassium hydrogen phosphate or the corresponding sodium-containing salts. The culture medium must also contain salts of metals, such as, 20 for example, magnesium sulfate or iron sulfate, which are necessary for growth. Finally, essential growth substances, such as amino acids and vitamins, may be used in addition to the above-mentioned substances. Suitable precursors may also be added to the culture medium. The mentioned 25 substances may be added to the culture in the form of a single batch, or they may be fed in in a suitable manner during the cultivation.

In order to control the pH value of the culture, basic compounds, such as sodium hydroxide, potassium hydroxide, 30 ammonia or ammonia water, or acid compounds, such as phosphoric acid or sulfuric acid, are expediently used. In order to control the development of foam, anti-foams, such as, for example, fatty acid polyglycol esters, may be used. In order to maintain the stability of plasmids, suitable 35 substances having a selective action, such as, for example,

antibiotics, may be added to the medium. In order to maintain aerobic conditions, oxygen or gas mixtures containing oxygen, such as, for example, air, are introduced into the culture. The temperature of the culture
5 is normally from 20°C to 45°C and preferably from 25°C to 40°C. The culture is continued until the maximum amount of the desired product has formed. That aim is normally achieved within a period of from 10 hours to 160 hours.

Methods of determining L-amino acids are known from the prior art. The analysis may be carried out, for example, as
10 described in Spackman et al. (Analytical Chemistry, 30, (1958), 1190) by ion-exchange chromatography with subsequent ninhydrin derivatization, or it may be carried out by reversed phase HPLC, as described in Lindroth et al.
15 (Analytical Chemistry (1979) 51: 1167-1174).

Pure cultures of the following microorganisms were deposited on 16 January 2001 at the Deutsche Sammlung für Mikroorganismen und Zellkulturen (DSMZ, Braunschweig, Germany) in accordance with the Budapest Treaty:

- 20 ◦ *Corynebacterium glutamicum* strain DM1546 as DSM 13993
- *Corynebacterium glutamicum* strain DM1547 as DSM 13994.

The process of the invention is used for the production of amino acids by fermentation.

The present invention is explained in greater detail below
25 by means of Examples.

The isolation of plasmid DNA from *Escherichia coli* and all techniques for restriction, Klenow and alkaline phosphatase treatment were carried out according to Sambrook et al. (Molecular Cloning. A Laboratory Manual (1989) Cold Spring
30 Harbour Laboratory Press, Cold Spring Harbor, NY, USA). Methods for the transformation of *Escherichia coli* are also described in that handbook.

The composition of common nutrient media, such as LB or TY medium, will also be found in the handbook of Sambrook et al..

Example 1

- 5 Preparation of a genomic cosmid gene library from
Corynebacterium glutamicum ATCC 13032

Chromosomal DNA from Corynebacterium glutamicum ATCC 13032 is isolated as described in Tauch et al. (1995, Plasmid 33:168-179) and partially cleaved with the restriction
10 enzyme Sau3AI (Amersham Pharmacia, Freiburg, Germany, product description Sau3AI, Code no. 27-0913-02). The DNA fragments are dephosphorylated with shrimp alkaline phosphatase (Roche Diagnostics GmbH, Mannheim, Germany, product description SAP, Code no. 1758250). The DNA of
15 cosmid vector SuperCos1 (Wahl et al. (1987) Proceedings of the National Academy of Sciences USA 84:2160-2164), obtained from Stratagene (La Jolla, USA, product description SuperCos1 Cosmid Vektor Kit, Code no. 251301), is cleaved with the restriction enzyme XbaI (Amersham
20 Pharmacia, Freiburg, Germany, product description XbaI, Code no. 27-0948-02) and likewise dephosphorylated with shrimp alkaline phosphatase.

The cosmid DNA is then cleaved with the restriction enzyme BamHI (Amersham Pharmacia, Freiburg, Germany, product
25 description BamHI, Code no. 27-0868-04). The cosmid DNA so treated is mixed with the treated ATCC13032 DNA, and the batch is treated with T4-DNA ligase (Amersham Pharmacia, Freiburg, Germany, product description T4-DNA ligase, Code no. 27-0870-04). The ligation mixture is then packed in
30 phages with the aid of Gigapack II XL Packing Extract (Stratagene, La Jolla, USA, product description Gigapack II XL Packing Extract, Code no. 200217).

For infection of *E. coli* strain NM554 (Raleigh et al. 1988, Nucleic Acid Research 16:1563-1575), the cells are taken up in 10 mM MgSO₄ and mixed with an aliquot of the phage suspension. Infection and titration of the cosmid library are carried out as described in Sambrook et al. (1989, Molecular Cloning: A Laboratory Manual, Cold Spring Harbor), the cells being plated out on LB agar (Lennox, 1955, Virology, 1:190) with 100 mg/l ampicillin. After incubation overnight at 37°C, recombinant individual clones are selected.

Example 2

Isolation and sequencing of the *rpoB* gene

The cosmid DNA of an individual colony is isolated using the Qiaprep Spin Miniprep Kit (Product No. 27106, Qiagen, Hilden, Germany) according to the manufacturer's instructions, and partially cleaved with the restriction enzyme Sau3AI (Amersham Pharmacia, Freiburg, Germany, product description Sau3AI, Product No. 27-0913-02). The DNA fragments are dephosphorylated with shrimp alkaline phosphatase (Roche Diagnostics GmbH, Mannheim, Germany, product description SAP, Product No. 1758250). After separation by gel electrophoresis, cosmid fragments having a size in the range from 1500 to 2000 bp are isolated using the QiaExII Gel Extraction Kit (Product No. 20021, Qiagen, Hilden, Germany).

The DNA of sequencing vector pZero-1, obtained from Invitrogen (Groningen, Netherlands, product description Zero Background Cloning Kit, Product No. K2500-01), is cleaved with the restriction enzyme BamHI (Amersham Pharmacia, Freiburg, Germany, product description BamHI, Product No. 27-0868-04). Ligation of the cosmid fragments into the sequencing vector pZero-1 is carried out as described by Sambrook et al. (1989, Molecular Cloning: A Laboratory Manual, Cold Spring Harbor), the DNA mixture

being incubated overnight with T4 ligase (Pharmacia Biotech, Freiburg, Germany). The ligation mixture is then electroporated into E. coli strain DH5 α MCR (Grant, 1990, Proceedings of the National Academy of Sciences U.S.A., 87:4645-4649) (Tauch et al. 1994, FEMS Microbiol Letters, 123:343-347) and plated out on LB agar (Lennox, 1955, Virology, 1:190) with 50 mg/l Zeocin.

Plasmid preparation of the recombinant clones is carried out using the Biorobot 9600 (Product No. 900200, Qiagen, Hilden, Germany). Sequencing is effected by the dideoxy chain termination method of Sanger et al. (1977, Proceedings of the National Academy of Sciences U.S.A., 74:5463-5467) with modifications according to Zimmermann et al. (1990, Nucleic Acids Research, 18:1067). The "RR dRhodamin Terminator Cycle Sequencing Kit" from PE Applied Biosystems (Product No. 403044, Weiterstadt, Germany) is used. Separation by gel electrophoresis and analysis of the sequencing reaction is carried out in a "Rotiphorese NF Acrylamid/Bisacrylamid" gel (29:1) (Product No. A124.1, Roth, Karlsruhe, Germany) using the "ABI Prism 377" sequencing device from PE Applied Biosystems (Weiterstadt, Germany).

The resulting crude sequence data are then processed using the Staden program package (1986, Nucleic Acids Research, 14:217-231) Version 97-0. The individual sequences of the pZero1 derivatives are assembled to a coherent contig. The computer-assisted coding region analysis is prepared using the program XNIP (Staden, 1986, Nucleic Acids Research, 14:217-231).

The resulting nucleotide sequence is shown in SEQ ID No. 1. Analysis of the nucleotide sequence gives an open reading frame of 3497 base pairs, which is designated the rpoB gene. The rpoB gene codes for a protein of 1165 amino acids.

SEQUENCE LISTING

<110> Degussa-Hüls AG

5 <120> Novel nucleotide sequences coding for the rpoB gene

<130> 000781 BT

<140>

10 <141>

<160> 8

<170> PatentIn Ver. 2.1

15

<210> 1

<211> 5099

<212> DNA

<213> Corynebacterium glutamicum

20

<220>

<221> CDS

<222> (702)..(4196)

<223> rpoB wild-type gene

25

<400> 1

acaatgtgac tcgtgatttt tgggtggatc agcgtaccgg tttggttgtc gatctagctg 60

aaaatattga tgattttttac ggcgaccgca gcggccagaa gtacgaacag aaattgcttt 120

30

tcgacgcctc cctcgacgat gcagctgtct ctaagctggg tgcacaggcc gaaagcatcc 180

ctgatggaga tgtgagcaaa atcgcaaata ccgtaggtat tgtgatcggg gcggatttgg 240

35

ctctcgtggg cctggccggg tgttttgggg cgtttgggaa gaaacgtcga gaagcttaac 300

ctgctgttca aatagatttt cctgttttcg aattgcggaa accccggggtt tgtttgctag 360

gggtgcctcgt agaaggggtc aagaagattt ctgggaaacg cgcccggtcg gttggttgct 420

40

aatagcacgc ggagcaccag atgaaaaatc tcccctttac tttcgcgcgc gattggtata 480

ctctgagtcg ttgcgttgga attcgtgact ctttttcggt cctgtagcgc caagaccttg 540

45

atcaaggtgg tttaaaaaaa ccgatttgac aagggtcattc agtgctatct ggagtcgttc 600

agggggatcg gggttcctcag cagaccaatt gctcaaaaat accagcgggtg ttgatctgca 660

cttaatggcc ttgaccagcc aggtgcaatt acccgcggtga g gtg ctg gaa gga ccc 716

50

Met Leu Glu Gly Pro
1 5atc ttg gca gtc tcc cgc cag acc aag tca gtc gtc gat att ccc ggt 764
Ile Leu Ala Val Ser Arg Gln Thr Lys Ser Val Val Asp Ile Pro Gly

55

10

15

20

gca ccg cag cgt tat tct ttc gcg aag gtg tcc gca ccc att gag gtg 812

	Ala	Pro	Gln	Arg	Tyr	Ser	Phe	Ala	Lys	Val	Ser	Ala	Pro	Ile	Glu	Val	
				25					30					35			
5	ccc	ggg	cta	cta	gat	ctt	caa	ctg	gat	tct	tac	tcc	tgg	ctg	att	ggg	860
	Pro	Gly	Leu	Leu	Asp	Leu	Gln	Leu	Asp	Ser	Tyr	Ser	Trp	Leu	Ile	Gly	
			40				45					50					
10	acg	cct	gag	tgg	cgt	gct	cgt	cag	aag	gaa	gaa	ttc	ggc	gag	gga	gcc	908
	Thr	Pro	Glu	Trp	Arg	Ala	Arg	Gln	Lys	Glu	Glu	Phe	Gly	Glu	Gly	Ala	
		55				60						65					
15	cgc	gta	acc	agc	ggc	ctt	gag	aac	att	ctc	gag	gag	ctc	tcc	cca	atc	956
	Arg	Val	Thr	Ser	Gly	Leu	Glu	Asn	Ile	Leu	Glu	Glu	Leu	Ser	Pro	Ile	
	70				75					80						85	
	cag	gat	tac	tct	gga	aac	atg	tcc	ctg	agc	ctt	tcg	gag	cca	cgc	ttc	1004
	Gln	Asp	Tyr	Ser	Gly	Asn	Met	Ser	Leu	Ser	Leu	Ser	Glu	Pro	Arg	Phe	
					90			95							100		
20	gaa	gac	gtc	aag	aac	acc	att	gac	gag	gcg	aaa	gaa	aag	gac	atc	aac	1052
	Glu	Asp	Val	Lys	Asn	Thr	Ile	Asp	Glu	Ala	Lys	Glu	Lys	Asp	Ile	Asn	
				105				110						115			
25	tac	gcg	gcg	cca	ctg	tat	gtg	acc	gcg	gag	ttc	gtc	aac	aac	acc	acc	1100
	Tyr	Ala	Ala	Pro	Leu	Tyr	Val	Thr	Ala	Glu	Phe	Val	Asn	Asn	Thr	Thr	
				120				125					130				
30	ggg	gaa	atc	aag	tct	cag	act	gtc	ttc	atc	ggc	gat	ttc	cca	atg	atg	1148
	Gly	Glu	Ile	Lys	Ser	Gln	Thr	Val	Phe	Ile	Gly	Asp	Phe	Pro	Met	Met	
		135				140					145						
35	acg	gac	aag	gga	acg	ttc	atc	atc	aac	gga	acc	gaa	cgc	gtt	gtg	gtc	1196
	Thr	Asp	Lys	Gly	Thr	Phe	Ile	Ile	Asn	Gly	Thr	Glu	Arg	Val	Val	Val	
		150				155					160					165	
	agc	cag	ctc	gtc	cgc	tcc	ccg	ggc	gtg	tac	ttt	gac	cag	acc	atc	gat	1244
	Ser	Gln	Leu	Val	Arg	Ser	Pro	Gly	Val	Tyr	Phe	Asp	Gln	Thr	Ile	Asp	
					170				175						180		
40	aag	tca	act	gag	cgt	cca	ctg	cac	gcc	gtg	aag	gtt	att	cct	tcc	cgt	1292
	Lys	Ser	Thr	Glu	Arg	Pro	Leu	His	Ala	Val	Lys	Val	Ile	Pro	Ser	Arg	
				185				190						195			
45	ggg	gct	tgg	ctt	gag	ttt	gac	gtc	gat	aag	cgc	gat	tcg	gtt	ggg	gtt	1340
	Gly	Ala	Trp	Leu	Glu	Phe	Asp	Val	Asp	Lys	Arg	Asp	Ser	Val	Gly	Val	
			200					205					210				
50	cgt	att	gac	cgc	aag	cgt	cgc	cag	cca	gtc	acc	gta	ctg	ctg	aag	gct	1388
	Arg	Ile	Asp	Arg	Lys	Arg	Arg	Gln	Pro	Val	Thr	Val	Leu	Leu	Lys	Ala	
		215				220						225					
55	ctt	ggc	tgg	acc	act	gag	cag	atc	acc	gag	cgt	ttc	ggg	ttc	tct	gaa	1436
	Leu	Gly	Trp	Thr	Thr	Glu	Gln	Ile	Thr	Glu	Arg	Phe	Gly	Phe	Ser	Glu	
		230				235					240					245	
	atc	atg	atg	tcc	acc	ctc	gag	tcc	gat	ggg	gta	gca	aac	acc	gat	gag	1484
	Ile	Met	Met	Ser	Thr	Leu	Glu	Ser	Asp	Gly	Val	Ala	Asn	Thr	Asp	Glu	
					250				255						260		

		gca	ttg	ctg	gag	atc	tac	cgc	aag	cag	cgt	cca	ggc	gag	cag	cct	acc	1532
		Ala	Leu	Leu	Glu	Ile	Tyr	Arg	Lys	Gln	Arg	Pro	Gly	Glu	Gln	Pro	Thr	
					265					270					275			
5		cgc	gac	ctt	gcg	cag	tcc	ctc	ctg	gac	aac	agc	ttc	ttc	cgt	gca	aag	1580
		Arg	Asp	Leu	Ala	Gln	Ser	Leu	Leu	Asp	Asn	Ser	Phe	Phe	Arg	Ala	Lys	
				280					285					290				
10		cgc	tac	gac	ctg	gct	cgc	gtt	ggc	cgt	tac	aag	atc	aac	cgc	aag	ctc	1628
		Arg	Tyr	Asp	Leu	Ala	Arg	Val	Gly	Arg	Tyr	Lys	Ile	Asn	Arg	Lys	Leu	
			295					300					305					
15		ggc	ctt	ggc	ggc	gac	cac	gat	ggc	ttg	atg	act	ctt	act	gaa	gag	gac	1676
		Gly	Leu	Gly	Gly	Asp	His	Asp	Gly	Leu	Met	Thr	Leu	Thr	Glu	Glu	Asp	
		310					315					320					325	
20		atc	gca	acc	acc	atc	gag	tac	ctg	gtg	cgt	ctg	cac	gca	ggc	gag	cgc	1724
		Ile	Ala	Thr	Thr	Ile	Glu	Tyr	Leu	Val	Arg	Leu	His	Ala	Gly	Glu	Arg	
						330					335					340		
25		gtc	atg	act	tct	cca	aac	ggc	gaa	gag	atc	cca	gtc	gag	acc	gat	gac	1772
		Val	Met	Thr	Ser	Pro	Asn	Gly	Glu	Glu	Ile	Pro	Val	Glu	Thr	Asp	Asp	
					345					350					355			
30		atc	gac	cac	ttt	ggc	aac	cgt	cgt	ctg	cgt	acc	gtt	ggc	gaa	ctg	atc	1820
		Ile	Asp	His	Phe	Gly	Asn	Arg	Arg	Leu	Arg	Thr	Val	Gly	Glu	Leu	Ile	
				360				365						370				
35		cag	aac	cag	gtc	cgt	gtc	ggc	ctg	tcc	cgc	atg	gag	cgc	gtt	gtt	cgt	1868
		Gln	Asn	Gln	Val	Arg	Val	Gly	Leu	Ser	Arg	Met	Glu	Arg	Val	Val	Arg	
			375					380					385					
40		gag	cgt	atg	acc	acc	cag	gat	gcg	gag	tcc	att	act	cct	act	tcc	ttg	1916
		Glu	Arg	Met	Thr	Thr	Gln	Asp	Ala	Glu	Ser	Ile	Thr	Pro	Thr	Ser	Leu	
		390					395					400					405	
45		atc	aac	gtt	cgt	cct	gtc	tct	gca	gct	atc	cgt	gag	ttc	ttc	gga	act	1964
		Ile	Asn	Val	Arg	Pro	Val	Ser	Ala	Ala	Ile	Arg	Glu	Phe	Phe	Gly	Thr	
						410					415					420		
50		tcc	cag	ctg	tct	cag	ttc	atg	gac	cag	aac	aac	tcc	ctg	tct	ggc	ttg	2012
		Ser	Gln	Leu	Ser	Gln	Phe	Met	Asp	Gln	Asn	Asn	Ser	Leu	Ser	Gly	Leu	
					425				430						435			
55		act	cac	aag	cgt	cgt	ctg	tcg	gct	ctg	ggc	ccg	ggc	ggc	ctg	tcc	cgt	2060
		Thr	His	Lys	Arg	Arg	Leu	Ser	Ala	Leu	Gly	Pro	Gly	Gly	Leu	Ser	Arg	
				440				445					450					
60		gag	cgc	gcc	ggc	atc	gag	gtt	cga	gac	gtt	cac	cca	tct	cac	tac	ggc	2108
		Glu	Arg	Ala	Gly	Ile	Glu	Val	Arg	Asp	Val	His	Pro	Ser	His	Tyr	Gly	
			455					460					465					
65		cgt	atg	tgc	cca	att	gag	act	ccg	gaa	ggc	cca	aac	att	ggc	ctg	atc	2156
		Arg	Met	Cys	Pro	Ile	Glu	Thr	Pro	Glu	Gly	Pro	Asn	Ile	Gly	Leu	Ile	
		470					475					480					485	
70		ggc	tcc	ttg	gct	tcc	tat	gct	cga	gtg	aac	cca	ttc	ggc	ttc	att	gag	2204
		Gly	Ser	Leu	Ala	Ser	Tyr	Ala	Arg	Val	Asn	Pro	Phe	Gly	Phe	Ile	Glu	
						490					495					500		

	acc	cca	tac	cgt	cgc	atc	atc	gac	ggc	aag	ctg	acc	gac	cag	att	gac	2252
	Thr	Pro	Tyr	Arg	Arg	Ile	Ile	Asp	Gly	Lys	Leu	Thr	Asp	Gln	Ile	Asp	
				505					510					515			
5	tac	ctt	acc	gct	gat	gag	gaa	gac	cgc	ttc	gtt	gtt	gcg	cag	gca	aac	2300
	Tyr	Leu	Thr	Ala	Asp	Glu	Glu	Asp	Arg	Phe	Val	Val	Ala	Gln	Ala	Asn	
			520					525					530				
10	acg	cac	tac	gac	gaa	gag	ggc	aac	atc	acc	gat	gag	acc	gtc	act	gtt	2348
	Thr	His	Tyr	Asp	Glu	Glu	Gly	Asn	Ile	Thr	Asp	Glu	Thr	Val	Thr	Val	
		535					540					545					
15	cgt	ctg	aag	gac	ggc	gac	atc	gcc	atg	gtt	ggc	cgc	aac	gcg	gtt	gat	2396
	Arg	Leu	Lys	Asp	Gly	Asp	Ile	Ala	Met	Val	Gly	Arg	Asn	Ala	Val	Asp	
	550					555					560					565	
20	tac	atg	gac	gtt	tcc	cct	cgt	cag	atg	gtt	tct	gtt	ggt	acc	gcg	atg	2444
	Tyr	Met	Asp	Val	Ser	Pro	Arg	Gln	Met	Val	Ser	Val	Gly	Thr	Ala	Met	
					570					575					580		
25	att	cca	ttc	ctg	gag	cac	gac	gat	gct	aac	cgt	gca	ctg	atg	ggc	gcg	2492
	Ile	Pro	Phe	Leu	Glu	His	Asp	Asp	Ala	Asn	Arg	Ala	Leu	Met	Gly	Ala	
				585					590					595			
30	aac	atg	cag	aag	cag	gct	gtg	cca	ctg	att	cgt	gcc	gag	gct	cct	ttc	2540
	Asn	Met	Gln	Lys	Gln	Ala	Val	Pro	Leu	Ile	Arg	Ala	Glu	Ala	Pro	Phe	
			600					605					610				
35	gtg	ggc	acc	ggg	atg	gag	cag	cgc	gca	gca	tac	gac	gcc	ggc	gac	ctg	2588
	Val	Gly	Thr	Gly	Met	Glu	Gln	Arg	Ala	Ala	Tyr	Asp	Ala	Gly	Asp	Leu	
		615					620					625					
40	gtt	att	acc	cca	gtc	gca	ggg	gtg	gtg	gaa	aac	gtt	tca	gct	gac	ttc	2636
	Val	Ile	Thr	Pro	Val	Ala	Gly	Val	Val	Glu	Asn	Val	Ser	Ala	Asp	Phe	
		630				635					640					645	
45	atc	acc	atc	atg	gct	gat	gac	ggc	aag	cgc	gaa	acc	tac	ctg	ctg	cgt	2684
	Ile	Thr	Ile	Met	Ala	Asp	Asp	Gly	Lys	Arg	Glu	Thr	Tyr	Leu	Leu	Arg	
				650						655					660		
50	aag	ttc	cag	cgc	acc	aac	cag	ggc	acc	agc	tac	aac	cag	aag	cct	ttg	2732
	Lys	Phe	Gln	Arg	Thr	Asn	Gln	Gly	Thr	Ser	Tyr	Asn	Gln	Lys	Pro	Leu	
				665					670					675			
55	gtt	aac	ttg	ggc	gag	cgc	gtt	gaa	gct	ggc	cag	gtt	att	gct	gat	ggg	2780
	Val	Asn	Leu	Gly	Glu	Arg	Val	Glu	Ala	Gly	Gln	Val	Ile	Ala	Asp	Gly	
			680					685					690				
60	cca	ggg	acc	ttc	aat	ggg	gaa	atg	tcc	ctt	ggc	cgt	aac	ctt	ctg	gtt	2828
	Pro	Gly	Thr	Phe	Asn	Gly	Glu	Met	Ser	Leu	Gly	Arg	Asn	Leu	Leu	Val	
		695					700					705					
65	gcg	ttc	atg	cct	tgg	gaa	ggc	cac	aac	tac	gag	gat	gcg	atc	atc	ctc	2876
	Ala	Phe	Met	Pro	Trp	Glu	Gly	His	Asn	Tyr	Glu	Asp	Ala	Ile	Ile	Leu	
		710					715				720					725	
70	aac	cag	aac	atc	gtt	gag	cag	gac	atc	ttg	acc	tcg	atc	cac	atc	gag	2924
	Asn	Gln	Asn	Ile	Val	Glu	Gln	Asp	Ile	Leu	Thr	Ser	Ile	His	Ile	Glu	

	730								735				740					
5	gag	cac	gag	atc	gat	gcc	cgc	gac	act	aag	ctt	ggc	gcc	gaa	gaa	atc	2972	
	Glu	His	Glu	Ile	Asp	Ala	Arg	Asp	Thr	Lys	Leu	Gly	Ala	Glu	Glu	Ile		
				745					750					755				
10	acc	cgc	gac	atc	cct	aat	gtg	tct	gaa	gaa	gtc	ctc	aag	gac	ctc	gac	3020	
	Thr	Arg	Asp	Ile	Pro	Asn	Val	Ser	Glu	Glu	Val	Leu	Lys	Asp	Leu	Asp		
				760				765					770					
15	gac	cgc	ggg	att	gtc	cgc	atc	ggg	gct	gat	gtt	cgt	gac	ggc	gac	atc	3068	
	Asp	Arg	Gly	Ile	Val	Arg	Ile	Gly	Ala	Asp	Val	Arg	Asp	Gly	Asp	Ile		
				775				780				785						
20	ctg	gtc	ggg	aag	gtc	acc	cct	aag	ggc	gag	acc	gag	ctc	acc	ccg	gaa	3116	
	Leu	Val	Gly	Lys	Val	Thr	Pro	Lys	Gly	Glu	Thr	Glu	Leu	Thr	Pro	Glu		
						795					800					805		
25	gag	cgc	ttg	ctg	cgc	gca	atc	ttc	ggg	gag	aag	gcc	cgc	gaa	gtt	cgc	3164	
	Glu	Arg	Leu	Leu	Arg	Ala	Ile	Phe	Gly	Glu	Lys	Ala	Arg	Glu	Val	Arg		
						810				815					820			
30	gat	acc	tcc	atg	aag	gtg	cct	cac	ggg	gag	acc	ggc	aag	gtc	atc	ggc	3212	
	Asp	Thr	Ser	Met	Lys	Val	Pro	His	Gly	Glu	Thr	Gly	Lys	Val	Ile	Gly		
						825				830				835				
35	gtg	cgt	cac	ttc	tcc	cgc	gag	gac	gac	gac	gat	ctg	gct	cct	ggc	gtc	3260	
	Val	Arg	His	Phe	Ser	Arg	Glu	Asp	Asp	Asp	Asp	Leu	Ala	Pro	Gly	Val		
						840			845				850					
40	aac	gag	atg	atc	cgt	atc	tac	gtt	gct	cag	aag	cgt	aag	atc	cag	gac	3308	
	Asn	Glu	Met	Ile	Arg	Ile	Tyr	Val	Ala	Gln	Lys	Arg	Lys	Ile	Gln	Asp		
							860					865						
45	ggc	gat	aag	ctc	gct	ggc	cgc	cac	ggg	aac	aag	ggg	gtt	gtc	ggg	aaa	3356	
	Gly	Asp	Lys	Leu	Ala	Gly	Arg	His	Gly	Asn	Lys	Gly	Val	Val	Gly	Lys		
						875					880					885		
50	att	ttg	cct	cag	gaa	gat	atg	cca	ttc	ctt	cca	gac	ggc	act	cct	gtt	3404	
	Ile	Leu	Pro	Gln	Glu	Asp	Met	Pro	Phe	Leu	Pro	Asp	Gly	Thr	Pro	Val		
						890				895					900			
55	gac	atc	atc	ttg	aac	acc	cac	ggg	gtt	cca	cgt	cgt	atg	aac	att	ggg	3452	
	Asp	Ile	Ile	Leu	Asn	Thr	His	Gly	Val	Pro	Arg	Arg	Met	Asn	Ile	Gly		
						905			910					915				
60	cag	gtt	ctt	gag	acc	cac	ctt	ggc	tgg	ctg	gca	tct	gct	ggg	tgg	tcc	3500	
	Gln	Val	Leu	Glu	Thr	His	Leu	Gly	Trp	Leu	Ala	Ser	Ala	Gly	Trp	Ser		
								925					930					
65	gtg	gat	cct	gaa	gat	cct	gag	aac	gct	gag	ctc	gtc	aag	act	ctg	cct	3548	
	Val	Asp	Pro	Glu	Asp	Pro	Glu	Asn	Ala	Glu	Leu	Val	Lys	Thr	Leu	Pro		
								940				945						
70	gca	gac	ctc	ctc	gag	gtt	cct	gct	ggg	tcc	ttg	act	gca	act	cct	gtg	3596	
	Ala	Asp	Leu	Leu	Glu	Val	Pro	Ala	Gly	Ser	Leu	Thr	Ala	Thr	Pro	Val		
						955					960					965		
75	ttc	gac	ggg	gcg	tca	aac	gaa	gag	ctc	gca	ggc	ctg	ctc	gct	aat	tca	3644	

55

gcggttaagta caagcgtgtc cgctacaagg gcatcatctg tgaacgctgt ggcgttgagg 4526
 tcaccaagtc caaggtgcgc cgtgagcgca tgggacacat tgagctcgct gcaccagtaa 4586
 5 cccacatttg gtacttcaag ggcgttccat cagcctcgg ctaccttttg gaccttgctc 4646
 caaaggacct ggacctcatc atctacttcg gtgcgaacat catcaccagc gtggacgaag 4706
 10 aggetcgcca cagcgaccag accactcttg aggcagaaat gcttctggag aagaaggacg 4766
 ttgaggcaga cgcagagtct gacattgctg agcgtgctga aaagctcgaa gaggatcttg 4826
 ctgaacttga ggcagctggc gctaaggccg acgctcgccg caaggttcag gctgctgccg 4886
 15 ataaggaaat gcagcacatc cgtgagcgtg cacagcgca aatcgatcgt ctcgatgagg 4946
 tctggcagac cttcatcaag cttgctccaa agcagatgat ccgcatgag aagctctacg 5006
 atgaactgat cgaccgctac gaggattact tcaccggtgg tatgggtgca gagtccattg 5066
 20 aggctttgat ccagaacttc gaccttgatg ctg 5099

<210> 2
 25 <211> 1165
 <212> PRT
 <213> Corynebacterium glutamicum

 <400> 2
 30 Met Leu Glu Gly Pro Ile Leu Ala Val Ser Arg Gln Thr Lys Ser Val
 1 5 10 15
 Val Asp Ile Pro Gly Ala Pro Gln Arg Tyr Ser Phe Ala Lys Val Ser
 20 25 30
 35 Ala Pro Ile Glu Val Pro Gly Leu Leu Asp Leu Gln Leu Asp Ser Tyr
 35 40 45
 Ser Trp Leu Ile Gly Thr Pro Glu Trp Arg Ala Arg Gln Lys Glu Glu
 40 50 55 60
 Phe Gly Glu Gly Ala Arg Val Thr Ser Gly Leu Glu Asn Ile Leu Glu
 65 70 75 80
 45 Glu Leu Ser Pro Ile Gln Asp Tyr Ser Gly Asn Met Ser Leu Ser Leu
 85 90 95
 Ser Glu Pro Arg Phe Glu Asp Val Lys Asn Thr Ile Asp Glu Ala Lys
 100 105 110
 50 Glu Lys Asp Ile Asn Tyr Ala Ala Pro Leu Tyr Val Thr Ala Glu Phe
 115 120 125
 Val Asn Asn Thr Thr Gly Glu Ile Lys Ser Gln Thr Val Phe Ile Gly
 55 130 135 140
 Asp Phe Pro Met Met Thr Asp Lys Gly Thr Phe Ile Ile Asn Gly Thr
 145 150 155 160

Glu Arg Val Val Val Ser Gln Leu Val Arg Ser Pro Gly Val Tyr Phe
 165 170 175
 5 Asp Gln Thr Ile Asp Lys Ser Thr Glu Arg Pro Leu His Ala Val Lys
 180 185 190
 Val Ile Pro Ser Arg Gly Ala Trp Leu Glu Phe Asp Val Asp Lys Arg
 195 200 205
 10 Asp Ser Val Gly Val Arg Ile Asp Arg Lys Arg Arg Gln Pro Val Thr
 210 215 220
 Val Leu Leu Lys Ala Leu Gly Trp Thr Thr Glu Gln Ile Thr Glu Arg
 225 230 235 240
 15 Phe Gly Phe Ser Glu Ile Met Met Ser Thr Leu Glu Ser Asp Gly Val
 245 250 255
 Ala Asn Thr Asp Glu Ala Leu Leu Glu Ile Tyr Arg Lys Gln Arg Pro
 260 265 270
 20 Gly Glu Gln Pro Thr Arg Asp Leu Ala Gln Ser Leu Leu Asp Asn Ser
 275 280 285
 25 Phe Phe Arg Ala Lys Arg Tyr Asp Leu Ala Arg Val Gly Arg Tyr Lys
 290 295 300
 Ile Asn Arg Lys Leu Gly Leu Gly Gly Asp His Asp Gly Leu Met Thr
 305 310 315 320
 30 Leu Thr Glu Glu Asp Ile Ala Thr Thr Ile Glu Tyr Leu Val Arg Leu
 325 330 335
 35 His Ala Gly Glu Arg Val Met Thr Ser Pro Asn Gly Glu Glu Ile Pro
 340 345 350
 Val Glu Thr Asp Asp Ile Asp His Phe Gly Asn Arg Arg Leu Arg Thr
 355 360 365
 40 Val Gly Glu Leu Ile Gln Asn Gln Val Arg Val Gly Leu Ser Arg Met
 370 375 380
 Glu Arg Val Val Arg Glu Arg Met Thr Thr Gln Asp Ala Glu Ser Ile
 385 390 395 400
 45 Thr Pro Thr Ser Leu Ile Asn Val Arg Pro Val Ser Ala Ala Ile Arg
 405 410 415
 50 Glu Phe Phe Gly Thr Ser Gln Leu Ser Gln Phe Met Asp Gln Asn Asn
 420 425 430
 Ser Leu Ser Gly Leu Thr His Lys Arg Arg Leu Ser Ala Leu Gly Pro
 435 440 445
 55 Gly Gly Leu Ser Arg Glu Arg Ala Gly Ile Glu Val Arg Asp Val His
 450 455 460
 Pro Ser His Tyr Gly Arg Met Cys Pro Ile Glu Thr Pro Glu Gly Pro
 465 470 475 480

	Asn	Ile	Gly	Leu	Ile	Gly	Ser	Leu	Ala	Ser	Tyr	Ala	Arg	Val	Asn	Pro	
					485					490					495		
5	Phe	Gly	Phe	Ile	Glu	Thr	Pro	Tyr	Arg	Arg	Ile	Ile	Asp	Gly	Lys	Leu	
				500					505					510			
	Thr	Asp	Gln	Ile	Asp	Tyr	Leu	Thr	Ala	Asp	Glu	Glu	Asp	Arg	Phe	Val	
			515					520					525				
10	Val	Ala	Gln	Ala	Asn	Thr	His	Tyr	Asp	Glu	Glu	Gly	Asn	Ile	Thr	Asp	
		530					535					540					
	Glu	Thr	Val	Thr	Val	Arg	Leu	Lys	Asp	Gly	Asp	Ile	Ala	Met	Val	Gly	
15	545					550					555					560	
	Arg	Asn	Ala	Val	Asp	Tyr	Met	Asp	Val	Ser	Pro	Arg	Gln	Met	Val	Ser	
					565					570					575		
20	Val	Gly	Thr	Ala	Met	Ile	Pro	Phe	Leu	Glu	His	Asp	Asp	Ala	Asn	Arg	
				580					585					590			
	Ala	Leu	Met	Gly	Ala	Asn	Met	Gln	Lys	Gln	Ala	Val	Pro	Leu	Ile	Arg	
			595					600					605				
25	Ala	Glu	Ala	Pro	Phe	Val	Gly	Thr	Gly	Met	Glu	Gln	Arg	Ala	Ala	Tyr	
		610					615					620					
	Asp	Ala	Gly	Asp	Leu	Val	Ile	Thr	Pro	Val	Ala	Gly	Val	Val	Glu	Asn	
30	625					630					635					640	
	Val	Ser	Ala	Asp	Phe	Ile	Thr	Ile	Met	Ala	Asp	Asp	Gly	Lys	Arg	Glu	
					645					650					655		
35	Thr	Tyr	Leu	Leu	Arg	Lys	Phe	Gln	Arg	Thr	Asn	Gln	Gly	Thr	Ser	Tyr	
				660					665					670			
	Asn	Gln	Lys	Pro	Leu	Val	Asn	Leu	Gly	Glu	Arg	Val	Glu	Ala	Gly	Gln	
			675					680					685				
40	Val	Ile	Ala	Asp	Gly	Pro	Gly	Thr	Phe	Asn	Gly	Glu	Met	Ser	Leu	Gly	
		690					695					700					
	Arg	Asn	Leu	Leu	Val	Ala	Phe	Met	Pro	Trp	Glu	Gly	His	Asn	Tyr	Glu	
45	705					710					715					720	
	Asp	Ala	Ile	Ile	Leu	Asn	Gln	Asn	Ile	Val	Glu	Gln	Asp	Ile	Leu	Thr	
					725					730					735		
50	Ser	Ile	His	Ile	Glu	Glu	His	Glu	Ile	Asp	Ala	Arg	Asp	Thr	Lys	Leu	
				740					745					750			
	Gly	Ala	Glu	Glu	Ile	Thr	Arg	Asp	Ile	Pro	Asn	Val	Ser	Glu	Glu	Val	
			755					760					765				
55	Leu	Lys	Asp	Leu	Asp	Asp	Arg	Gly	Ile	Val	Arg	Ile	Gly	Ala	Asp	Val	
		770					775					780					
	Arg	Asp	Gly	Asp	Ile	Leu	Val	Gly	Lys	Val	Thr	Pro	Lys	Gly	Glu	Thr	

	785		790		795		800									
	Glu	Leu	Thr	Pro	Glu	Glu	Arg	Leu	Leu	Arg	Ala	Ile	Phe	Gly	Glu	Lys
					805					810					815	
5	Ala	Arg	Glu	Val	Arg	Asp	Thr	Ser	Met	Lys	Val	Pro	His	Gly	Glu	Thr
				820					825					830		
10	Gly	Lys	Val	Ile	Gly	Val	Arg	His	Phe	Ser	Arg	Glu	Asp	Asp	Asp	Asp
			835					840					845			
	Leu	Ala	Pro	Gly	Val	Asn	Glu	Met	Ile	Arg	Ile	Tyr	Val	Ala	Gln	Lys
		850					855					860				
15	Arg	Lys	Ile	Gln	Asp	Gly	Asp	Lys	Leu	Ala	Gly	Arg	His	Gly	Asn	Lys
	865					870					875				880	
	Gly	Val	Val	Gly	Lys	Ile	Leu	Pro	Gln	Glu	Asp	Met	Pro	Phe	Leu	Pro
					885					890					895	
20	Asp	Gly	Thr	Pro	Val	Asp	Ile	Ile	Leu	Asn	Thr	His	Gly	Val	Pro	Arg
				900					905					910		
	Arg	Met	Asn	Ile	Gly	Gln	Val	Leu	Glu	Thr	His	Leu	Gly	Trp	Leu	Ala
25			915					920					925			
	Ser	Ala	Gly	Trp	Ser	Val	Asp	Pro	Glu	Asp	Pro	Glu	Asn	Ala	Glu	Leu
		930					935					940				
30	Val	Lys	Thr	Leu	Pro	Ala	Asp	Leu	Leu	Glu	Val	Pro	Ala	Gly	Ser	Leu
	945					950					955					960
	Thr	Ala	Thr	Pro	Val	Phe	Asp	Gly	Ala	Ser	Asn	Glu	Glu	Leu	Ala	Gly
				965						970					975	
35	Leu	Leu	Ala	Asn	Ser	Arg	Pro	Asn	Arg	Asp	Gly	Asp	Val	Met	Val	Asn
				980					985					990		
	Ala	Asp	Gly	Lys	Ala	Thr	Leu	Ile	Asp	Gly	Arg	Ser	Gly	Glu	Pro	Tyr
40			995					1000					1005			
	Pro	Tyr	Pro	Val	Ser	Ile	Gly	Tyr	Met	Tyr	Met	Leu	Lys	Leu	His	His
		1010					1015					1020				
45	Leu	Val	Asp	Glu	Lys	Ile	His	Ala	Arg	Ser	Thr	Gly	Pro	Tyr	Ser	Met
	025					1030					1035				1040	
	Ile	Thr	Gln	Gln	Pro	Leu	Gly	Gly	Lys	Ala	Gln	Phe	Gly	Gly	Gln	Arg
				1045					1050						1055	
50	Phe	Gly	Glu	Met	Glu	Val	Trp	Ala	Met	Gln	Ala	Tyr	Gly	Ala	Ala	Tyr
			1060					1065					1070			
	Thr	Leu	Gln	Glu	Leu	Leu	Thr	Ile	Lys	Ser	Asp	Asp	Val	Val	Gly	Arg
55		1075						1080					1085			
	Val	Lys	Val	Tyr	Glu	Ala	Ile	Val	Lys	Gly	Glu	Asn	Ile	Pro	Asp	Pro
		1090					1095					1100				

Gly Ile Pro Glu Ser Phe Lys Val Leu Leu Lys Glu Leu Gln Ser Leu
 105 1110 1115 1120
 5 Cys Leu Asn Val Glu Val Leu Ser Ala Asp Gly Thr Pro Met Glu Leu
 1125 1130 1135
 Ala Gly Asp Asp Asp Asp Phe Asp Gln Ala Gly Ala Ser Leu Gly Ile
 1140 1145 1150
 10 Asn Leu Ser Arg Asp Glu Arg Ser Asp Ala Asp Thr Ala
 1155 1160 1165
 15 <210> 3
 <211> 5099
 <212> DNA
 <213> Corynebacterium glutamicum
 20 <220>
 <221> CDS
 <222> (702)..(4196)
 <223> rpoB gene allele 1547
 25 <400> 3
 acaatgtgac tcgtgatttt tgggtggatc agcgtaccgg tttggttgtc gatctagctg 60
 aaaatattga tgattttttac ggcgaccgca gcggccagaa gtacgaacag aaattgcttt 120
 30 tcgacgcctc cctcgacgat gcagctgtct ctaagctggg tgcacaggcc gaaagcatcc 180
 ctgatggaga tgtgagcaaa atcgcaaata ccgtaggtat tgtgatcggg gcggtattgg 240
 35 ctctcgtggg cctggccggg tgttttgggg cgtttgggaa gaaacgtcga gaagcttaac 300
 ctgctgttca aatagatttt ccctgtttcg aattgcggaa accccggggt tgtttgctag 360
 ggtgcctcgt agaaggggtc aagaagattt ctgggaaacg cgcccggtgc gttggttgct 420
 40 aatagcacgc ggagcaccag atgaaaaatc tcccctttac tttcgcgcgc gattggtata 480
 ctctgagtcg ttgcgttgga attcgtgact ctttttcggt cctgtagcgc caagaccttg 540
 atcaaggtgg tttaaaaaaa ccgatttgac aagggtcattc agtgctatct ggagtcgttc 600
 45 aggggggatcg ggttcctcag cagaccaatt gctcaaaaat accagcgggt ttgatctgca 660
 cttaatggcc ttgaccagcc aggtgcaatt acccgcggtga g gtg ctg gaa gga ctc 716
 Met Leu Glu Gly Leu
 50 1 5
 atc ttg gca gtc tcc cgc cag acc aag tca gtc gtc gat att ccc ggt 764
 Ile Leu Ala Val Ser Arg Gln Thr Lys Ser Val Val Asp Ile Pro Gly
 10 15 20
 55 gca ccg cag cgt tat tct ttc gcg aag gtg tcc gca ccc att gag gtg 812
 Ala Pro Gln Arg Tyr Ser Phe Ala Lys Val Ser Ala Pro Ile Glu Val
 25 30 35

	ccc ggg cta cta gat ctt caa ctg gat tct tac tcc tgg ctg att ggt	860
	Pro Gly Leu Leu Asp Leu Gln Leu Asp Ser Tyr Ser Trp Leu Ile Gly	
	40 45 50	
5	acg cct gag tgg cgt gct cgt cag aag gaa gaa ttc ggc gag gga gcc	908
	Thr Pro Glu Trp Arg Ala Arg Gln Lys Glu Glu Phe Gly Glu Gly Ala	
	55 60 65	
10	cgc gta acc agc ggc ctt gag aac att ctc gag gag ctc tcc cca atc	956
	Arg Val Thr Ser Gly Leu Glu Asn Ile Leu Glu Glu Leu Ser Pro Ile	
	70 75 80 85	
15	cag gat tac tct gga aac atg tcc ctg agc ctt tcg gag cca cgc ttc	1004
	Gln Asp Tyr Ser Gly Asn Met Ser Leu Ser Leu Ser Glu Pro Arg Phe	
	90 95 100	
20	gaa gac gtc aag aac acc att gac gag gcg aaa gaa aag gac atc aac	1052
	Glu Asp Val Lys Asn Thr Ile Asp Glu Ala Lys Glu Lys Asp Ile Asn	
	105 110 115	
25	tac gcg gcg cca ctg tat gtg acc gcg gag ttc gtc aac aac acc acc	1100
	Tyr Ala Ala Pro Leu Tyr Val Thr Ala Glu Phe Val Asn Asn Thr Thr	
	120 125 130	
30	ggt gaa atc aag tct cag act gtc ttc atc ggc gat ttc cca atg atg	1148
	Gly Glu Ile Lys Ser Gln Thr Val Phe Ile Gly Asp Phe Pro Met Met	
	135 140 145	
35	acg gac aag gga acg ttc atc atc aac gga acc gaa cgc gtt gtg gtc	1196
	Thr Asp Lys Gly Thr Phe Ile Ile Asn Gly Thr Glu Arg Val Val Val	
	150 155 160 165	
40	agc cag ctc gtc cgc tcc ccg ggc gtg tac ttt gac cag acc atc gat	1244
	Ser Gln Leu Val Arg Ser Pro Gly Val Tyr Phe Asp Gln Thr Ile Asp	
	170 175 180	
45	aag tca act gag cgt cca ctg cac gcc gtg aag gtt att cct ttc cgt	1292
	Lys Ser Thr Glu Arg Pro Leu His Ala Val Lys Val Ile Pro Phe Arg	
	185 190 195	
50	ggt gct tgg ctt gag ttt gac gtc gat aag cgc gat tcg gtt ggt gtt	1340
	Gly Ala Trp Leu Glu Phe Asp Val Asp Lys Arg Asp Ser Val Gly Val	
	200 205 210	
55	cgt att gac cgc aag cgt cgc cag cca gtc acc gta ctg ctg aag gct	1388
	Arg Ile Asp Arg Lys Arg Arg Gln Pro Val Thr Val Leu Leu Lys Ala	
	215 220 225	
60	ctt ggc tgg acc act gag cag atc acc gag cgt ttc ggt ttc tct gaa	1436
	Leu Gly Trp Thr Thr Glu Gln Ile Thr Glu Arg Phe Gly Phe Ser Glu	
	230 235 240 245	
65	atc atg atg tcc acc ctc gag tcc gat ggt gta gca aac acc gat gag	1484
	Ile Met Met Ser Thr Leu Glu Ser Asp Gly Val Ala Asn Thr Asp Glu	
	250 255 260	
70	gca ttg ctg gag atc tac cgc aag cag cgt cca ggc gag cag cct acc	1532
	Ala Leu Leu Glu Ile Tyr Arg Lys Gln Arg Pro Gly Glu Gln Pro Thr	
	265 270 275	

5	cgc gac ctt gcg cag tcc ctc ctg gac aac agc ttc ttc cgt gca aag	1580
	Arg Asp Leu Ala Gln Ser Leu Leu Asp Asn Ser Phe Phe Arg Ala Lys	
	280 285 290	
10	cgc tac gac ctg gct cgc gtt ggt cgt tac aag atc aac cgc aag ctc	1628
	Arg Tyr Asp Leu Ala Arg Val Gly Arg Tyr Lys Ile Asn Arg Lys Leu	
	295 300 305	
15	ggc ctt ggt ggc gac cac gat ggt ttg atg act ctt act gaa gag gac	1676
	Gly Leu Gly Gly Asp His Asp Gly Leu Met Thr Leu Thr Glu Glu Asp	
	310 315 320 325	
20	atc gca acc acc atc gag tac ctg gtg cgt ctg cac gca ggt gag cgc	1724
	Ile Ala Thr Thr Ile Glu Tyr Leu Val Arg Leu His Ala Gly Glu Arg	
	330 335 340	
25	gtc atg act tct cca aat ggt gaa gag atc cca gtc gag acc gat gac	1772
	Val Met Thr Ser Pro Asn Gly Glu Ile Pro Val Glu Thr Asp Asp	
	345 350 355	
30	atc gac cac ttt ggt aac cgt cgt ctg cgt acc gtt ggc gaa ctg atc	1820
	Ile Asp His Phe Gly Asn Arg Arg Leu Arg Thr Val Gly Glu Leu Ile	
	360 365 370	
35	cag aac cag gtc cgt gtc ggc ctg tcc cgc atg gag cgc gtt gtt cgt	1868
	Gln Asn Gln Val Arg Val Gly Leu Ser Arg Met Glu Arg Val Val Arg	
	375 380 385	
40	gag cgt atg acc acc cag gat gcg gag tcc att act cct act tcc ttg	1916
	Glu Arg Met Thr Thr Gln Asp Ala Glu Ser Ile Thr Pro Thr Ser Leu	
	390 395 400 405	
45	atc aac gtt cgt cct gtc tct gca gct atc cgt gag ttc ttc gga act	1964
	Ile Asn Val Arg Pro Val Ser Ala Ala Ile Arg Glu Phe Phe Gly Thr	
	410 415 420	
50	tcc cag ctg tct cag ttc atg gtc cag aac aac tcc ctg tct ggt ttg	2012
	Ser Gln Leu Ser Gln Phe Met Val Gln Asn Asn Ser Leu Ser Gly Leu	
	425 430 435	
55	act cac aag cgt cgt ctg tgc gct ctg ggc ccg ggt ggt ctg tcc cgt	2060
	Thr His Lys Arg Arg Leu Ser Ala Leu Gly Pro Gly Gly Leu Ser Arg	
	440 445 450	
60	gag cgc gcc ggc atc gag gtt cga gac gtt cac cca tct cac tac ggc	2108
	Glu Arg Ala Gly Ile Glu Val Arg Asp Val His Pro Ser His Tyr Gly	
	455 460 465	
65	cgt atg tgc cca att gag act ccg gaa ggt cca aac att ggc ctg atc	2156
	Arg Met Cys Pro Ile Glu Thr Pro Glu Gly Pro Asn Ile Gly Leu Ile	
	470 475 480 485	
70	ggc tcc ttg gct tcc tat gct cga gtg aac cca ttc ggt ttc att gag	2204
	Gly Ser Leu Ala Ser Tyr Ala Arg Val Asn Pro Phe Gly Phe Ile Glu	
	490 495 500	
75	acc cca tac cgt cgc atc atc gac ggc aag ctg acc gac cag att gac	2252
	Thr Pro Tyr Arg Arg Ile Ile Asp Gly Lys Leu Thr Asp Gln Ile Asp	

	505							510							515							
5	tac	ctt	acc	gct	gat	gag	gaa	gac	cgc	ttc	gtt	gtt	gcg	cag	gca	aac	2300					
	Tyr	Leu	Thr	Ala	Asp	Glu	Glu	Asp	Arg	Phe	Val	Val	Ala	Gln	Ala	Asn						
			520					525					530									
10	acg	cac	tac	gac	gaa	gag	ggc	aac	atc	acc	gat	gag	acc	gtc	act	gtt	2348					
	Thr	His	Tyr	Asp	Glu	Glu	Gly	Asn	Ile	Thr	Asp	Glu	Thr	Val	Thr	Val						
			535				540					545										
15	cgt	ctg	aag	gac	ggc	gac	atc	gcc	atg	gtt	ggc	cgc	aac	gcg	gtt	gat	2396					
	Arg	Leu	Lys	Asp	Gly	Asp	Ile	Ala	Met	Val	Gly	Arg	Asn	Ala	Val	Asp						
						555					560					565						
20	tac	atg	gac	gtt	tcc	cct	cgt	cag	atg	gtt	tct	gtt	ggc	acc	gcg	atg	2444					
	Tyr	Met	Asp	Val	Ser	Pro	Arg	Gln	Met	Val	Ser	Val	Gly	Thr	Ala	Met						
						570				575					580							
25	att	cca	ttc	ctg	gag	cac	gac	gat	gct	aac	cgt	gca	ctg	atg	ggc	gcg	2492					
	Ile	Pro	Phe	Leu	Glu	His	Asp	Asp	Ala	Asn	Arg	Ala	Leu	Met	Gly	Ala						
				585					590					595								
30	aac	atg	cag	aag	cag	gct	gtg	cca	ctg	att	cgt	gcc	gag	gct	cct	ttc	2540					
	Asn	Met	Gln	Lys	Gln	Ala	Val	Pro	Leu	Ile	Arg	Ala	Glu	Ala	Pro	Phe						
				600				605					610									
35	gtg	ggc	acc	ggt	atg	gag	cag	cgc	gca	gca	tac	gac	gcc	ggc	gac	ctg	2588					
	Val	Gly	Thr	Gly	Met	Glu	Gln	Arg	Ala	Ala	Tyr	Asp	Ala	Gly	Asp	Leu						
				615			620					625										
40	gtt	att	acc	cca	gtc	gca	ggt	gtg	gtg	gaa	aac	gtt	tca	gct	gac	ttc	2636					
	Val	Ile	Thr	Pro	Val	Ala	Gly	Val	Val	Glu	Asn	Val	Ser	Ala	Asp	Phe						
						635					640					645						
45	atc	acc	atc	atg	gct	gat	gac	ggc	aag	cgc	gaa	acc	tac	ctg	ctg	cgt	2684					
	Ile	Thr	Ile	Met	Ala	Asp	Asp	Gly	Lys	Arg	Glu	Thr	Tyr	Leu	Leu	Arg						
					650					655					660							
50	aag	ttc	cag	cgc	acc	aac	cag	ggc	acc	agc	tac	aac	cag	aag	cct	ttg	2732					
	Lys	Phe	Gln	Arg	Thr	Asn	Gln	Gly	Thr	Ser	Tyr	Asn	Gln	Lys	Pro	Leu						
				665				670						675								
55	gtt	aac	ttg	ggc	gag	cgc	gtt	gaa	gct	ggc	cag	gtt	att	gct	gat	ggt	2780					
	Val	Asn	Leu	Gly	Glu	Arg	Val	Glu	Ala	Gly	Gln	Val	Ile	Ala	Asp	Gly						
				680				685					690									
60	cca	ggt	acc	ttc	aat	ggt	gaa	atg	tcc	ctt	ggc	cgt	aac	ctt	ctg	gtt	2828					
	Pro	Gly	Thr	Phe	Asn	Gly	Glu	Met	Ser	Leu	Gly	Arg	Asn	Leu	Leu	Val						
				695			700					705										
65	gcg	ttc	atg	cct	tgg	gaa	ggc	cac	aac	tac	gag	gat	gcg	atc	atc	ctc	2876					
	Ala	Phe	Met	Pro	Trp	Glu	Gly	His	Asn	Tyr	Glu	Asp	Ala	Ile	Ile	Leu						
						715					720					725						
70	aac	cag	aac	atc	gtt	gag	cag	gac	atc	ttg	acc	tcg	atc	cac	atc	gag	2924					
	Asn	Gln	Asn	Ile	Val	Glu	Gln	Asp	Ile	Leu	Thr	Ser	Ile	His	Ile	Glu						
					730					735					740							
75	gag	cac	gag	atc	gat	gcc	cgc	gac	act	aag	ctt	ggc	gcc	gaa	gaa	atc	2972					

	Glu	His	Glu	Ile	Asp	Ala	Arg	Asp	Thr	Lys	Leu	Gly	Ala	Glu	Glu	Ile	
				745					750					755			
5	acc	cgc	gac	atc	cct	aat	gtg	tct	gaa	gaa	gtc	ctc	aag	gac	ctc	gac	3020
	Thr	Arg	Asp	Ile	Pro	Asn	Val	Ser	Glu	Glu	Val	Leu	Lys	Asp	Leu	Asp	
			760					765					770				
10	gac	cgc	ggg	att	gtc	cgc	atc	ggg	gct	gat	gtt	cgt	gac	ggc	gac	atc	3068
	Asp	Arg	Gly	Ile	Val	Arg	Ile	Gly	Ala	Asp	Val	Arg	Asp	Gly	Asp	Ile	
			775				780					785					
15	ctg	gtc	ggg	aag	gtc	acc	cct	aag	ggc	gag	acc	gag	ctc	acc	ccg	gaa	3116
	Leu	Val	Gly	Lys	Val	Thr	Pro	Lys	Gly	Glu	Thr	Glu	Leu	Thr	Pro	Glu	
	790					795					800					805	
	gag	cgc	ttg	ctg	cgc	gca	atc	ttc	ggg	gag	aag	gcc	cgc	gaa	gtt	cgc	3164
	Glu	Arg	Leu	Leu	Arg	Ala	Ile	Phe	Gly	Glu	Lys	Ala	Arg	Glu	Val	Arg	
					810				815					820			
20	gat	acc	tcc	atg	aag	gtg	cct	cac	ggg	gag	acc	ggc	aag	gtc	atc	ggc	3212
	Asp	Thr	Ser	Met	Lys	Val	Pro	His	Gly	Glu	Thr	Gly	Lys	Val	Ile	Gly	
				825					830					835			
25	gtg	cgt	cac	ttc	tcc	cgc	gag	gac	gac	gac	gat	ctg	gct	cct	ggc	gtc	3260
	Val	Arg	His	Phe	Ser	Arg	Glu	Asp	Asp	Asp	Asp	Leu	Ala	Pro	Gly	Val	
			840					845					850				
30	aac	gag	atg	atc	cgt	atc	tac	gtt	gct	cag	aag	cgt	aag	atc	cag	gac	3308
	Asn	Glu	Met	Ile	Arg	Ile	Tyr	Val	Ala	Gln	Lys	Arg	Lys	Ile	Gln	Asp	
		855					860					865					
35	ggc	gat	aag	ctc	gct	ggc	cgc	cac	ggg	aac	aag	ggg	gtt	gtc	ggg	aaa	3356
	Gly	Asp	Lys	Leu	Ala	Gly	Arg	His	Gly	Asn	Lys	Gly	Val	Val	Gly	Lys	
	870					875				880						885	
	att	ttg	cct	cag	gaa	gat	atg	cca	ttc	ctt	cca	gac	ggc	act	cct	gtt	3404
	Ile	Leu	Pro	Gln	Glu	Asp	Met	Pro	Phe	Leu	Pro	Asp	Gly	Thr	Pro	Val	
					890					895					900		
40	gac	atc	atc	ttg	aac	acc	cac	ggg	gtt	cca	cgt	cgt	atg	aac	att	ggg	3452
	Asp	Ile	Ile	Leu	Asn	Thr	His	Gly	Val	Pro	Arg	Arg	Met	Asn	Ile	Gly	
				905				910						915			
45	cag	gtt	ctt	gag	acc	cac	ctt	ggc	tgg	ctg	gca	tct	gct	ggg	tgg	tcc	3500
	Gln	Val	Leu	Glu	Thr	His	Leu	Gly	Trp	Leu	Ala	Ser	Ala	Gly	Trp	Ser	
			920					925					930				
50	gtg	gat	cct	gaa	gat	cct	gag	aac	gct	gag	ctc	gtc	aag	act	ctg	cct	3548
	Val	Asp	Pro	Glu	Asp	Pro	Glu	Asn	Ala	Glu	Leu	Val	Lys	Thr	Leu	Pro	
			935				940					945					
55	gca	gac	ctc	ctc	gag	gtt	cct	gct	ggg	tcc	ttg	act	gca	act	cct	gtg	3596
	Ala	Asp	Leu	Leu	Glu	Val	Pro	Ala	Gly	Ser	Leu	Thr	Ala	Thr	Pro	Val	
	950					955				960						965	
	ttc	gac	ggg	gag	tca	aac	gaa	gag	ctc	gca	ggc	ctg	ctc	gct	aat	tca	3644
	Phe	Asp	Gly	Ala	Ser	Asn	Glu	Glu	Leu	Ala	Gly	Leu	Leu	Ala	Asn	Ser	
					970					975					980		

cgt cca aac cgc gac ggc gac gtc atg gtt aac gcg gat ggt aaa gca 3692
 Arg Pro Asn Arg Asp Gly Asp Val Met Val Asn Ala Asp Gly Lys Ala
 985 990 995

5 acg ctt atc gac ggt cgc tcc ggt gag cct tac ccg tac ccg gtt tcc 3740
 Thr Leu Ile Asp Gly Arg Ser Gly Glu Pro Tyr Pro Tyr Pro Val Ser
 1000 1005 1010

10 atc ggc tac atg tac atg ctg aag ctg cac cac ctc gtt gac gag aag 3788
 Ile Gly Tyr Met Tyr Met Leu Lys Leu His His Leu Val Asp Glu Lys
 1015 1020 1025

15 atc cac gca cgt tcc act ggt cct tac tcc atg att acc cag cag cca 3836
 Ile His Ala Arg Ser Thr Gly Pro Tyr Ser Met Ile Thr Gln Gln Pro
 1030 1035 1040 1045

20 ctg ggt ggt aaa gca cag ttc ggt gga cag cgt ttc ggc gaa atg gag 3884
 Leu Gly Gly Lys Ala Gln Phe Gly Gly Gln Arg Phe Gly Glu Met Glu
 1050 1055 1060

25 gtg tgg gca atg cag gca tac ggc gct gcc tac aca ctt cag gag ctg 3932
 Val Trp Ala Met Gln Ala Tyr Gly Ala Ala Tyr Thr Leu Gln Glu Leu
 1065 1070 1075

30 ctg acc atc aag tct gat gac gtg gtt ggc cgt gtc aag gtc tac gaa 3980
 Leu Thr Ile Lys Ser Asp Asp Val Val Gly Arg Val Lys Val Tyr Glu
 1080 1085 1090

35 gca att gtg aag ggc gag aac atc ccg gat cca ggt att cct gag tcc 4028
 Ala Ile Val Lys Gly Glu Asn Ile Pro Asp Pro Gly Ile Pro Glu Ser
 1095 1100 1105

40 ttc aag gtt ctc ctc aag gag ctc cag tcc ttg tgc ctg aac gtg gag 4076
 Phe Lys Val Leu Leu Lys Glu Leu Gln Ser Leu Cys Leu Asn Val Glu
 1110 1115 1120 1125

45 gtt ctc tcc gca gac ggc act cca atg gag ctc gcg ggt gac gac gac 4124
 Val Leu Ser Ala Asp Gly Thr Pro Met Glu Leu Ala Gly Asp Asp Asp
 1130 1135 1140

50 gac ttc gat cag gca ggc gcc tca ctt ggc atc aac ctg tcc cgt gac 4172
 Asp Phe Asp Gln Ala Gly Ala Ser Leu Gly Ile Asn Leu Ser Arg Asp
 1145 1150 1155

55 gag cgt tcc gac gcc gac acc gca tagcagatca gaaaacaacc gctagaaatc 4226
 Glu Arg Ser Asp Ala Asp Thr Ala
 1160 1165

aagccatata tcccccgac attgaagaga tggtctgggg ggaaaggag ttttacgtgc 4286

tcgacgtaaa cgtcttcgat gagctccgca tcggcctggc caccgccgac gacatccgcc 4346

gttggtccaa ggggtgaggtc aagaagccgg agaccatcaa ctaccgaacc ctcaagcctg 4406

agaaggacgg tctgttctgc gagcgtatct tcgggtccaac tcgcgactgg gagtgcgctt 4466

gcggttaagta caagcgtgtc cgctacaagg gcatcatctg tgaacgctgt ggcgttgagg 4526

tcaccaagtc caaggtgcgc cgtgagcgca tgggacacat tgagctcgct gcaccagtaa 4586

```

cccacatttg gtacttcaag ggcgttccat cagcctcgg ctaccttttg gaccttgctc 4646
caaaggacct ggacctcatc atctacttcg gtgcgaacat catcaccagc gtggacgaag 4706
5 aggctcgcca cagcgaccag accactcttg aggagaaat gcttctggag aagaaggacg 4766
ttgaggcaga cgcagagtct gacattgctg agcgtgctga aaagctcgaa gaggatcttg 4826
10 ctgaacttga ggcagctggc gctaaggccg acgctcgccg caagggttcag gctgctgccg 4886
ataaggaaat gcagcacatc cgtgagcgtg cacagcgcg aatcgatcgt ctcgatgagg 4946
tctggcagac cttcatcaag cttgctccaa agcagatgat ccgcatgag aagctctacg 5006
15 atgaactgat cgaccgctac gaggattact tcaccggtgg tatgggtgca gagtccattg 5066
aggctttgat ccagaacttc gaccttgatg ctg 5099

20
<210> 4
<211> 1165
<212> PRT
<213> Corynebacterium glutamicum

25
<400> 4
Met Leu Glu Gly Leu Ile Leu Ala Val Ser Arg Gln Thr Lys Ser Val
  1          5          10          15

30 Val Asp Ile Pro Gly Ala Pro Gln Arg Tyr Ser Phe Ala Lys Val Ser
      20          25          30

Ala Pro Ile Glu Val Pro Gly Leu Leu Asp Leu Gln Leu Asp Ser Tyr
      35          40          45

35 Ser Trp Leu Ile Gly Thr Pro Glu Trp Arg Ala Arg Gln Lys Glu Glu
      50          55          60

Phe Gly Glu Gly Ala Arg Val Thr Ser Gly Leu Glu Asn Ile Leu Glu
40      65          70          75          80

Glu Leu Ser Pro Ile Gln Asp Tyr Ser Gly Asn Met Ser Leu Ser Leu
      85          90          95

45 Ser Glu Pro Arg Phe Glu Asp Val Lys Asn Thr Ile Asp Glu Ala Lys
      100          105          110

Glu Lys Asp Ile Asn Tyr Ala Ala Pro Leu Tyr Val Thr Ala Glu Phe
      115          120          125

50 Val Asn Asn Thr Thr Gly Glu Ile Lys Ser Gln Thr Val Phe Ile Gly
      130          135          140

Asp Phe Pro Met Met Thr Asp Lys Gly Thr Phe Ile Ile Asn Gly Thr
55      145          150          155          160

Glu Arg Val Val Val Ser Gln Leu Val Arg Ser Pro Gly Val Tyr Phe
      165          170          175

```

Asp Gln Thr Ile Asp Lys Ser Thr Glu Arg Pro Leu His Ala Val Lys
 180 185 190
 5 Val Ile Pro Phe Arg Gly Ala Trp Leu Glu Phe Asp Val Asp Lys Arg
 195 200 205
 Asp Ser Val Gly Val Arg Ile Asp Arg Lys Arg Arg Gln Pro Val Thr
 210 215 220
 10 Val Leu Leu Lys Ala Leu Gly Trp Thr Thr Glu Gln Ile Thr Glu Arg
 225 230 235 240
 Phe Gly Phe Ser Glu Ile Met Met Ser Thr Leu Glu Ser Asp Gly Val
 245 250 255
 15 Ala Asn Thr Asp Glu Ala Leu Leu Glu Ile Tyr Arg Lys Gln Arg Pro
 260 265 270
 20 Gly Glu Gln Pro Thr Arg Asp Leu Ala Gln Ser Leu Leu Asp Asn Ser
 275 280 285
 Phe Phe Arg Ala Lys Arg Tyr Asp Leu Ala Arg Val Gly Arg Tyr Lys
 290 295 300
 25 Ile Asn Arg Lys Leu Gly Leu Gly Gly Asp His Asp Gly Leu Met Thr
 305 310 315 320
 Leu Thr Glu Glu Asp Ile Ala Thr Thr Ile Glu Tyr Leu Val Arg Leu
 325 330 335
 30 His Ala Gly Glu Arg Val Met Thr Ser Pro Asn Gly Glu Glu Ile Pro
 340 345 350
 35 Val Glu Thr Asp Asp Ile Asp His Phe Gly Asn Arg Arg Leu Arg Thr
 355 360 365
 Val Gly Glu Leu Ile Gln Asn Gln Val Arg Val Gly Leu Ser Arg Met
 370 375 380
 40 Glu Arg Val Val Arg Glu Arg Met Thr Thr Gln Asp Ala Glu Ser Ile
 385 390 395 400
 Thr Pro Thr Ser Leu Ile Asn Val Arg Pro Val Ser Ala Ala Ile Arg
 405 410 415
 45 Glu Phe Phe Gly Thr Ser Gln Leu Ser Gln Phe Met Val Gln Asn Asn
 420 425 430
 50 Ser Leu Ser Gly Leu Thr His Lys Arg Arg Leu Ser Ala Leu Gly Pro
 435 440 445
 Gly Gly Leu Ser Arg Glu Arg Ala Gly Ile Glu Val Arg Asp Val His
 450 455 460
 55 Pro Ser His Tyr Gly Arg Met Cys Pro Ile Glu Thr Pro Glu Gly Pro
 465 470 475 480
 Asn Ile Gly Leu Ile Gly Ser Leu Ala Ser Tyr Ala Arg Val Asn Pro
 485 490 495

	Phe	Gly	Phe	Ile	Glu	Thr	Pro	Tyr	Arg	Arg	Ile	Ile	Asp	Gly	Lys	Leu	
				500					505					510			
5	Thr	Asp	Gln	Ile	Asp	Tyr	Leu	Thr	Ala	Asp	Glu	Glu	Asp	Arg	Phe	Val	
			515					520					525				
	Val	Ala	Gln	Ala	Asn	Thr	His	Tyr	Asp	Glu	Glu	Gly	Asn	Ile	Thr	Asp	
		530					535					540					
10	Glu	Thr	Val	Thr	Val	Arg	Leu	Lys	Asp	Gly	Asp	Ile	Ala	Met	Val	Gly	
	545					550					555					560	
	Arg	Asn	Ala	Val	Asp	Tyr	Met	Asp	Val	Ser	Pro	Arg	Gln	Met	Val	Ser	
15					565					570					575		
	Val	Gly	Thr	Ala	Met	Ile	Pro	Phe	Leu	Glu	His	Asp	Asp	Ala	Asn	Arg	
				580					585					590			
20	Ala	Leu	Met	Gly	Ala	Asn	Met	Gln	Lys	Gln	Ala	Val	Pro	Leu	Ile	Arg	
			595					600					605				
	Ala	Glu	Ala	Pro	Phe	Val	Gly	Thr	Gly	Met	Glu	Gln	Arg	Ala	Ala	Tyr	
		610					615					620					
25	Asp	Ala	Gly	Asp	Leu	Val	Ile	Thr	Pro	Val	Ala	Gly	Val	Val	Glu	Asn	
	625					630					635					640	
	Val	Ser	Ala	Asp	Phe	Ile	Thr	Ile	Met	Ala	Asp	Asp	Gly	Lys	Arg	Glu	
30					645					650					655		
	Thr	Tyr	Leu	Leu	Arg	Lys	Phe	Gln	Arg	Thr	Asn	Gln	Gly	Thr	Ser	Tyr	
				660					665					670			
35	Asn	Gln	Lys	Pro	Leu	Val	Asn	Leu	Gly	Glu	Arg	Val	Glu	Ala	Gly	Gln	
			675					680					685				
	Val	Ile	Ala	Asp	Gly	Pro	Gly	Thr	Phe	Asn	Gly	Glu	Met	Ser	Leu	Gly	
		690					695					700					
40	Arg	Asn	Leu	Leu	Val	Ala	Phe	Met	Pro	Trp	Glu	Gly	His	Asn	Tyr	Glu	
	705					710					715					720	
	Asp	Ala	Ile	Ile	Leu	Asn	Gln	Asn	Ile	Val	Glu	Gln	Asp	Ile	Leu	Thr	
45					725					730					735		
	Ser	Ile	His	Ile	Glu	Glu	His	Glu	Ile	Asp	Ala	Arg	Asp	Thr	Lys	Leu	
				740					745					750			
50	Gly	Ala	Glu	Glu	Ile	Thr	Arg	Asp	Ile	Pro	Asn	Val	Ser	Glu	Glu	Val	
			755					760					765				
	Leu	Lys	Asp	Leu	Asp	Asp	Arg	Gly	Ile	Val	Arg	Ile	Gly	Ala	Asp	Val	
		770					775					780					
55	Arg	Asp	Gly	Asp	Ile	Leu	Val	Gly	Lys	Val	Thr	Pro	Lys	Gly	Glu	Thr	
	785					790					795					800	
	Glu	Leu	Thr	Pro	Glu	Glu	Arg	Leu	Leu	Arg	Ala	Ile	Phe	Gly	Glu	Lys	

	805										810					815				
	Ala	Arg	Glu	Val	Arg	Asp	Thr	Ser	Met	Lys	Val	Pro	His	Gly	Glu	Thr				
				820					825					830						
5	Gly	Lys	Val	Ile	Gly	Val	Arg	His	Phe	Ser	Arg	Glu	Asp	Asp	Asp	Asp				
			835					840					845							
10	Leu	Ala	Pro	Gly	Val	Asn	Glu	Met	Ile	Arg	Ile	Tyr	Val	Ala	Gln	Lys				
		850					855					860								
	Arg	Lys	Ile	Gln	Asp	Gly	Asp	Lys	Leu	Ala	Gly	Arg	His	Gly	Asn	Lys				
	865					870					875				880					
15	Gly	Val	Val	Gly	Lys	Ile	Leu	Pro	Gln	Glu	Asp	Met	Pro	Phe	Leu	Pro				
					885					890					895					
	Asp	Gly	Thr	Pro	Val	Asp	Ile	Ile	Leu	Asn	Thr	His	Gly	Val	Pro	Arg				
20				900					905					910						
	Arg	Met	Asn	Ile	Gly	Gln	Val	Leu	Glu	Thr	His	Leu	Gly	Trp	Leu	Ala				
			915					920					925							
25	Ser	Ala	Gly	Trp	Ser	Val	Asp	Pro	Glu	Asp	Pro	Glu	Asn	Ala	Glu	Leu				
		930					935					940								
	Val	Lys	Thr	Leu	Pro	Ala	Asp	Leu	Leu	Glu	Val	Pro	Ala	Gly	Ser	Leu				
	945					950					955					960				
30	Thr	Ala	Thr	Pro	Val	Phe	Asp	Gly	Ala	Ser	Asn	Glu	Glu	Leu	Ala	Gly				
					965					970					975					
	Leu	Leu	Ala	Asn	Ser	Arg	Pro	Asn	Arg	Asp	Gly	Asp	Val	Met	Val	Asn				
35				980					985					990						
	Ala	Asp	Gly	Lys	Ala	Thr	Leu	Ile	Asp	Gly	Arg	Ser	Gly	Glu	Pro	Tyr				
			995					1000					1005							
40	Pro	Tyr	Pro	Val	Ser	Ile	Gly	Tyr	Met	Tyr	Met	Leu	Lys	Leu	His	His				
		1010					1015					1020								
	Leu	Val	Asp	Glu	Lys	Ile	His	Ala	Arg	Ser	Thr	Gly	Pro	Tyr	Ser	Met				
	025					1030				1035					1040					
45	Ile	Thr	Gln	Gln	Pro	Leu	Gly	Gly	Lys	Ala	Gln	Phe	Gly	Gly	Gln	Arg				
					1045					1050					1055					
	Phe	Gly	Glu	Met	Glu	Val	Trp	Ala	Met	Gln	Ala	Tyr	Gly	Ala	Ala	Tyr				
50				1060					1065					1070						
	Thr	Leu	Gln	Glu	Leu	Leu	Thr	Ile	Lys	Ser	Asp	Asp	Val	Val	Gly	Arg				
				1075				1080					1085							
55	Val	Lys	Val	Tyr	Glu	Ala	Ile	Val	Lys	Gly	Glu	Asn	Ile	Pro	Asp	Pro				
		1090					1095					1100								
	Gly	Ile	Pro	Glu	Ser	Phe	Lys	Val	Leu	Leu	Lys	Glu	Leu	Gln	Ser	Leu				
	105					1110					1115				1120					

Cys Leu Asn Val Glu Val Leu Ser Ala Asp Gly Thr Pro Met Glu Leu
1125 1130 1135

Ala Gly Asp Asp Asp Asp Phe Asp Gln Ala Gly Ala Ser Leu Gly Ile
1140 1145 1150

Asn Leu Ser Arg Asp Glu Arg Ser Asp Ala Asp Thr Ala
1155 1160 1165

10

<210> 5
<211> 5099
<212> DNA
15 <213> Corynebacterium glutamicum

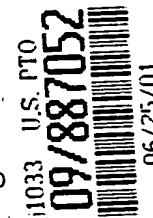
<220>
<221> CDS
<222> (702)..(4196)
20 <223> rpoB gene allele 1546

<400> 5
acaatgtgac tcgtgatttt tgggtggatc agcgtaccgg tttggttgct gatctagctg 60
25 aaaatattga tgatttttac ggcgaccgca gcggccagaa gtacgaacag aaattgcttt 120
tcgaagcctc cctcgacgat gcagctgtct ctaagctggg tgcacaggcc gaaagcatcc 180
ctgatggaga tgtgagcaaa atcgcaaata ccgtaggtat tgtgatcggt gcggtattgg 240
30 ctctcgtagg cctggccggg tgttttgggg cgtttgggaa gaaacgtcga gaagcttaac 300
ctgctgttca aatagatttt ccctgtttcg aattgcggaa accccggggt tgtttgctag 360
35 ggtgcctcgt agaaggggtc aagaagattt ctgggaaacg cgcccgtgct gttggttgct 420
aatagcacgc ggagcaccag atgaaaaatc tcccctttac ttctcgcgcg gattgggtata 480
ctctgagtcg ttgcgttgga attcgtgact ctttttcggt cctgtagcgc caagaccttg 540
40 atcaaggtgg tttaaaaaaa ccgatttgac aaggtcattc agtgctatct ggagtcgttc 600
aggggggatcg ggttcctcag cagaccaatt gctcaaaaat accagcgggtg ttgatctgca 660
45 cttaatggcc ttgaccagcc aggtgcaatt acccgcggtga g gtg ctg gaa gga ccc 716
Met Leu Glu Gly Pro
1 5
atc ttg gca gtc tcc cgc cag acc aag tca gtc gtc gat att ccc ggt 764
50 Ile Leu Ala Val Ser Arg Gln Thr Lys Ser Val Val Asp Ile Pro Gly
10 15 20
gca ccg cag cgt tat tct ttc gcg aag gtg tcc gca ccc att gag gtg 812
Ala Pro Gln Arg Tyr Ser Phe Ala Lys Val Ser Ala Pro Ile Glu Val
25 30 35
ccc ggg cta cta gat ctt caa ctg gat tct tac tcc tgg ctg att ggt 860
Pro Gly Leu Leu Asp Leu Gln Leu Asp Ser Tyr Ser Trp Leu Ile Gly
40 45 50

	acg cct gag tgg cgt gct cgt cag aag gaa gaa ttc ggc gag gga gcc	908
	Thr Pro Glu Trp Arg Ala Arg Gln Lys Glu Glu Phe Gly Glu Gly Ala	
	55 60 65	
5	cgc gta acc agc ggc ctt gag aac att ctc gag gag ctc tcc cca atc	956
	Arg Val Thr Ser Gly Leu Glu Asn Ile Leu Glu Glu Leu Ser Pro Ile	
	70 75 80 85	
10	cag gat tac tct gga aac atg tcc ctg agc ctt tcg gag cca cgc ttc	1004
	Gln Asp Tyr Ser Gly Asn Met Ser Leu Ser Leu Ser Glu Pro Arg Phe	
	90 95 100	
15	gaa gac gtc aag aac acc att gac gag gcg aaa gaa aag gac atc aac	1052
	Glu Asp Val Lys Asn Thr Ile Asp Glu Ala Lys Glu Lys Asp Ile Asn	
	105 110 115	
20	tac gcg gcg cca ctg tat gtg acc gcg gag ttc gtc aac aac acc acc	1100
	Tyr Ala Ala Pro Leu Tyr Val Thr Ala Glu Phe Val Asn Asn Thr Thr	
	120 125 130	
25	ggc gaa atc aag tct cag act gtc ttc atc ggc gat ttc cca atg atg	1148
	Gly Glu Ile Lys Ser Gln Thr Val Phe Ile Gly Asp Phe Pro Met Met	
	135 140 145	
30	acg gac aag gga acg ttc atc atc aac gga acc gaa cgc gtt gtg gtc	1196
	Thr Asp Lys Gly Thr Phe Ile Ile Asn Gly Thr Glu Arg Val Val Val	
	150 155 160 165	
35	agc cag ctc gtc cgc tcc ccg ggc gtg tac ttt gac cag acc atc gat	1244
	Ser Gln Leu Val Arg Ser Pro Gly Val Tyr Phe Asp Gln Thr Ile Asp	
	170 175 180	
40	aag tca act gag cgt cca ctg cac gcc gtg aag gtt att cct tcc cgt	1292
	Lys Ser Thr Glu Arg Pro Leu His Ala Val Lys Val Ile Pro Ser Arg	
	185 190 195	
45	ggc gct tgg ctt gag ttt gac gtc gat aag cgc gat tcg gtt ggt gtt	1340
	Gly Ala Trp Leu Glu Phe Asp Val Asp Lys Arg Asp Ser Val Gly Val	
	200 205 210	
50	cgt att gac cgc aag cgt cgc cag cca gtc acc gta ctg ctg aag gct	1388
	Arg Ile Asp Arg Lys Arg Arg Gln Pro Val Thr Val Leu Leu Lys Ala	
	215 220 225	
55	ctt ggc tgg acc act gag cag atc acc gag cgt ttc ggt ttc tct gaa	1436
	Leu Gly Trp Thr Thr Glu Gln Ile Thr Glu Arg Phe Gly Phe Ser Glu	
	230 235 240 245	
60	atc atg atg tcc acc ctc gag tcc gat ggt gta gca aac acc gat gag	1484
	Ile Met Met Ser Thr Leu Glu Ser Asp Gly Val Ala Asn Thr Asp Glu	
	250 255 260	
65	gca ttg ctg gag atc tac cgc aag cag cgt cca ggc gag cag cct acc	1532
	Ala Leu Leu Glu Ile Tyr Arg Lys Gln Arg Pro Gly Glu Gln Pro Thr	
	265 270 275	
70	cgc gac ctt gcg cag tcc ctc ctg gac aac agc ttc ttc cgt gca aag	1580
	Arg Asp Leu Ala Gln Ser Leu Leu Asp Asn Ser Phe Phe Arg Ala Lys	

	280	285	290	
5	cgc tac gac ctg gct cgc gtt ggt cgt tac aag atc aac cgc aag ctc Arg Tyr Asp Leu Ala Arg Val Gly Arg Tyr Lys Ile Asn Arg Lys Leu 295 300 305			1628
10	ggc ctt ggt ggc gac cac gat ggt ttg atg act ctt act gaa gag gac Gly Leu Gly Gly Asp His Asp Gly Leu Met Thr Leu Thr Glu Glu Asp 310 315 320 325			1676
	atc gca acc acc atc gag tac ctg gtg cgt ctg cac gca ggt gag cgc Ile Ala Thr Thr Ile Glu Tyr Leu Val Arg Leu His Ala Gly Glu Arg 330 335 340			1724
15	gtc atg act tct cca aat ggt gaa gag atc cca gtc gag acc gat gac Val Met Thr Ser Pro Asn Gly Glu Glu Ile Pro Val Glu Thr Asp Asp 345 350 355			1772
20	atc gac cac ttt ggt aac cgt cgt ctg cgt acc gtt ggc gaa ctg atc Ile Asp His Phe Gly Asn Arg Arg Leu Arg Thr Val Gly Glu Leu Ile 360 365 370			1820
25	cag aac cag gtc cgt gtc ggc ctg tcc cgc atg gag cgc gtt gtt cgt Gln Asn Gln Val Arg Val Gly Leu Ser Arg Met Glu Arg Val Val Arg 375 380 385			1868
30	gag cgt atg acc acc cag gat gcg gag tcc att act cct act tcc ttg Glu Arg Met Thr Thr Gln Asp Ala Glu Ser Ile Thr Pro Thr Ser Leu 390 395 400 405			1916
	atc aac gtt cgt cct gtc tct gca gct atc cgt gag ttc ttc gga act Ile Asn Val Arg Pro Val Ser Ala Ala Ile Arg Glu Phe Phe Gly Thr 410 415 420			1964
35	tcc cag ctg tct cag ttc atg gac cag aac aac tcc ctg tct ggt ttg Ser Gln Leu Ser Gln Phe Met Asp Gln Asn Asn Ser Leu Ser Gly Leu 425 430 435			2012
40	act tac aag cgt cgt ctg tcg gct ctg ggc ccg ggt ggt ctg tcc cgt Thr Tyr Lys Arg Arg Leu Ser Ala Leu Gly Pro Gly Gly Leu Ser Arg 440 445 450			2060
45	gag cgc gcc ggc atc gag gtt cga gac gtt cac cca tct cac tac ggc Glu Arg Ala Gly Ile Glu Val Arg Asp Val His Pro Ser His Tyr Gly 455 460 465			2108
50	cgt atg tgc cca att gag act ccg gaa ggt cca aac att ggc ctg atc Arg Met Cys Pro Ile Glu Thr Pro Glu Gly Pro Asn Ile Gly Leu Ile 470 475 480 485			2156
	ggc tcc ttg gct tcc tat gct cga gtg aac cca ttc ggt ttc att gag Gly Ser Leu Ala Ser Tyr Ala Arg Val Asn Pro Phe Gly Phe Ile Glu 490 495 500			2204
55	acc cca tac cgt cgc atc atc gac ggc aag ctg acc gac cag att gac Thr Pro Tyr Arg Arg Ile Ile Asp Gly Lys Leu Thr Asp Gln Ile Asp 505 510 515			2252

	tac	ctt	acc	gct	gat	gag	gaa	gac	cgc	ttc	gtt	gtt	gcg	cag	gca	aac	2300
	Tyr	Leu	Thr	Ala	Asp	Glu	Glu	Asp	Arg	Phe	Val	Val	Ala	Gln	Ala	Asn	
			520					525					530				
5	acg	cac	tac	gac	gaa	gag	ggc	aac	atc	acc	gat	gag	acc	gtc	act	gtt	2348
	Thr	His	Tyr	Asp	Glu	Glu	Gly	Asn	Ile	Thr	Asp	Glu	Thr	Val	Thr	Val	
		535					540					545					
10	cgt	ctg	aag	gac	ggc	gac	atc	gcc	atg	gtt	ggc	cgc	aac	gcg	gtt	gat	2396
	Arg	Leu	Lys	Asp	Gly	Asp	Ile	Ala	Met	Val	Gly	Arg	Asn	Ala	Val	Asp	
	550					555					560					565	
15	tac	atg	gac	gtt	tcc	cct	cgt	cag	atg	gtt	tct	gtt	ggt	acc	gcg	atg	2444
	Tyr	Met	Asp	Val	Ser	Pro	Arg	Gln	Met	Val	Ser	Val	Gly	Thr	Ala	Met	
					570					575					580		
20	att	cca	ttc	ctg	gag	cac	gac	gat	gct	aac	cgt	gca	ctg	atg	ggc	gcg	2492
	Ile	Pro	Phe	Leu	Glu	His	Asp	Asp	Ala	Asn	Arg	Ala	Leu	Met	Gly	Ala	
				585					590					595			
25	aac	atg	cag	aag	cag	gct	gtg	cca	ctg	att	cgt	gcc	gag	gct	cct	ttc	2540
	Asn	Met	Gln	Lys	Gln	Ala	Val	Pro	Leu	Ile	Arg	Ala	Glu	Ala	Pro	Phe	
			600					605					610				
30	gtg	ggc	acc	ggg	atg	gag	cag	cgc	gca	gca	tac	gac	gcc	ggc	gac	ctg	2588
	Val	Gly	Thr	Gly	Met	Glu	Gln	Arg	Ala	Ala	Tyr	Asp	Ala	Gly	Asp	Leu	
		615					620					625					
35	gtt	att	acc	cca	gtc	gca	ggg	gtg	gtg	gaa	aac	gtt	tca	gct	gac	ttc	2636
	Val	Ile	Thr	Pro	Val	Ala	Gly	Val	Val	Glu	Asn	Val	Ser	Ala	Asp	Phe	
	630					635					640					645	
40	atc	acc	atc	atg	gct	gat	gac	ggc	aag	cgc	gaa	acc	tac	ctg	ctg	cgt	2684
	Ile	Thr	Ile	Met	Ala	Asp	Asp	Gly	Lys	Arg	Glu	Thr	Tyr	Leu	Leu	Arg	
					650					655					660		
45	aag	ttc	cag	cgc	acc	aac	cag	ggc	acc	agc	tac	aac	cag	aag	cct	ttg	2732
	Lys	Phe	Gln	Arg	Thr	Asn	Gln	Gly	Thr	Ser	Tyr	Asn	Gln	Lys	Pro	Leu	
			665						670					675			
50	gtt	aac	ttg	ggc	gag	cgc	gtt	gaa	gct	ggc	cag	gtt	att	gct	gat	ggg	2780
	Val	Asn	Leu	Gly	Glu	Arg	Val	Glu	Ala	Gly	Gln	Val	Ile	Ala	Asp	Gly	
			680					685					690				
55	cca	ggg	acc	ttc	aat	ggg	gaa	atg	tcc	ctt	ggc	cgt	aac	ctt	ctg	gtt	2828
	Pro	Gly	Thr	Phe	Asn	Gly	Glu	Met	Ser	Leu	Gly	Arg	Asn	Leu	Leu	Val	
		695					700					705					
60	gcg	ttc	atg	cct	tgg	gaa	ggc	cac	aac	tac	gag	gat	gcg	atc	atc	ctc	2876
	Ala	Phe	Met	Pro	Trp	Glu	Gly	His	Asn	Tyr	Glu	Asp	Ala	Ile	Ile	Leu	
	710					715					720					725	
65	aac	cag	aac	atc	gtt	gag	cag	gac	atc	ttg	acc	tcg	atc	cac	atc	gag	2924
	Asn	Gln	Asn	Ile	Val	Glu	Gln	Asp	Ile	Leu	Thr	Ser	Ile	His	Ile	Glu	
					730					735					740		
70	gag	cac	gag	atc	gat	gcc	cgc	gac	act	aag	ctt	ggc	gcc	gaa	gaa	atc	2972
	Glu	His	Glu	Ile	Asp	Ala	Arg	Asp	Thr	Lys	Leu	Gly	Ala	Glu	Glu	Ile	
				745					750					755			



	acc cgc gac atc cct aat gtg tct gaa gaa gtc ctc aag gac ctc gac	3020
	Thr Arg Asp Ile Pro Asn Val Ser Glu Glu Val Leu Lys Asp Leu Asp	
	760 765 770	
5		
	gac cgc ggt att gtc cgc atc ggt gct gat gtt cgt gac ggc gac atc	3068
	Asp Arg Gly Ile Val Arg Ile Gly Ala Asp Val Arg Asp Gly Asp Ile	
	775 780 785	
10		
	ctg gtc ggt aag gtc acc cct aag ggc gag acc gag ctc acc ccg gaa	3116
	Leu Val Gly Lys Val Thr Pro Lys Gly Glu Thr Glu Leu Thr Pro Glu	
	790 795 800 805	
15		
	gag cgc ttg ctg cgc gca atc ttc ggt gag aag gcc cgc gaa gtt cgc	3164
	Glu Arg Leu Leu Arg Ala Ile Phe Gly Glu Lys Ala Arg Glu Val Arg	
	810 815 820	
20		
	gat acc tcc atg aag gtg cct cac ggt gag acc ggc aag gtc atc ggc	3212
	Asp Thr Ser Met Lys Val Pro His Gly Glu Thr Gly Lys Val Ile Gly	
	825 830 835	
25		
	gtg cgt cac ttc tcc cgc gag gac gac gac gat ctg gct cct ggc gtc	3260
	Val Arg His Phe Ser Arg Glu Asp Asp Asp Leu Ala Pro Gly Val	
	840 845 850	
30		
	aac gag atg atc cgt atc tac gtt gct cag aag cgt aag atc cag gac	3308
	Asn Glu Met Ile Arg Ile Tyr Val Ala Gln Lys Arg Lys Ile Gln Asp	
	855 860 865	
35		
	ggc gat aag ctc gct ggc cgc cac ggt aac aag ggt gtt gtc ggt aaa	3356
	Gly Asp Lys Leu Ala Gly Arg His Gly Asn Lys Gly Val Val Gly Lys	
	870 875 880 885	
40		
	att ttg cct cag gaa gat atg cca ttc ctt cca gac ggc act cct gtt	3404
	Ile Leu Pro Gln Glu Asp Met Pro Phe Leu Pro Asp Gly Thr Pro Val	
	890 895 900	
45		
	gac atc atc ttg aac acc cac ggt gtt cca cgt cgt atg aac att ggt	3452
	Asp Ile Ile Leu Asn Thr His Gly Val Pro Arg Arg Met Asn Ile Gly	
	905 910 915	
50		
	cag gtt ctt gag acc cac ctt ggc tgg ctg gca tct gct ggt tgg tcc	3500
	Gln Val Leu Glu Thr His Leu Gly Trp Leu Ala Ser Ala Gly Trp Ser	
	920 925 930	
55		
	gtg gat cct gaa gat cct gag aac gct gag ctc gtc aag act ctg cct	3548
	Val Asp Pro Glu Asp Pro Glu Asn Ala Glu Leu Val Lys Thr Leu Pro	
	935 940 945	
60		
	gca gac ctc ctc gag gtt cct gct ggt tcc ttg act gca act cct gtg	3596
	Ala Asp Leu Leu Glu Val Pro Ala Gly Ser Leu Thr Ala Thr Pro Val	
	950 955 960 965	
65		
	ttc gac ggt gcg tca aac gaa gag ctc gca ggc ctg ctc gct aat tca	3644
	Phe Asp Gly Ala Ser Asn Glu Glu Leu Ala Gly Leu Leu Ala Asn Ser	
	970 975 980	
70		
	cgt cca aac cgc gac ggc gac gtc atg gtt aac gcg gat ggt aaa gca	3692
	Arg Pro Asn Arg Asp Gly Asp Val Met Val Asn Ala Asp Gly Lys Ala	

	985	990	995	
5	acg ctt atc gac ggt cgc tcc ggt Thr Leu Ile Asp Gly Arg Ser Gly	gag cct tac ccg tac ccg gtt tcc Glu Pro Tyr Pro Tyr Pro Val Ser		3740
	1000	1005	1010	
10	atc ggc tac atg tac atg ctg aag ctg cac cac ctc gtt gac gag aag Ile Gly Tyr Met Tyr Met Leu Lys Leu His His Leu Val Asp Glu Lys			3788
	1015	1020	1025	
15	atc cac gca cgt tcc act ggt cct tac tcc atg att acc cag cag cca Ile His Ala Arg Ser Thr Gly Pro Tyr Ser Met Ile Thr Gln Gln Pro			3836
	1030	1035	1040	1045
20	ctg ggt ggt aaa gca cag ttc ggt gga cag cgt ttc ggc gaa atg gag Leu Gly Gly Lys Ala Gln Phe Gly Gly Gln Arg Phe Gly Glu Met Glu			3884
	1050	1055	1060	
25	gtg tgg gca atg cag gca tac ggc gct gcc tac aca ctt cag gag ctg Val Trp Ala Met Gln Ala Tyr Gly Ala Ala Tyr Thr Leu Gln Glu Leu			3932
	1065	1070	1075	
30	ctg acc atc aag tct gat gac gtg gtt ggc cgt gtc aag gtc tac gaa Leu Thr Ile Lys Ser Asp Asp Val Val Gly Arg Val Lys Val Tyr Glu			3980
	1080	1085	1090	
35	gca att gtg aag ggc gag aac atc ccg gat cca ggt att cct gag tcc Ala Ile Val Lys Gly Glu Asn Ile Pro Asp Pro Gly Ile Pro Glu Ser			4028
	1095	1100	1105	
40	ttc aag gtt ctc ctc aag gag ctc cag tcc ttg tgc ctg aac gtg gag Phe Lys Val Leu Leu Lys Glu Leu Gln Ser Leu Cys Leu Asn Val Glu			4076
	1110	1115	1120	1125
45	gtt ctc tcc gca gac ggc act cca atg gag ctc gcg ggt gac gac gac Val Leu Ser Ala Asp Gly Thr Pro Met Glu Leu Ala Gly Asp Asp Asp			4124
	1130	1135	1140	
50	gac ttc gat cag gca ggc gcc tca ctt ggc atc aac ctg tcc cgt gac Asp Phe Asp Gln Ala Gly Ala Ser Leu Gly Ile Asn Leu Ser Arg Asp			4172
	1145	1150	1155	
55	gag cgt tcc gac gcc gac acc gca tagcagatca gaaaacaacc gctagaaatc Glu Arg Ser Asp Ala Asp Thr Ala			4226
	1160	1165		
60	aagccatata tcccccgac attgaagaga tggtctgggg ggaaaggag ttttacgtgc			4286
65	tcgacgtaaa cgtcttcgat gagctccgca tcggcctggc caccgccgac gacatccgcc			4346
70	gttggtccaa ggggtgaggtc aagaagccgg agaccatcaa ctaccgaacc ctcaagcctg			4406
75	agaaggacgg tctgttctgc gagcgtatct tcgggtccaac tcgcgactgg gagtgcgctt			4466
80	gcggtaagta caagcgtgtc cgctacaagg gcatcatctg tgaacgctgt ggcgttgagg			4526
85	tcaccaagtc caaggtgctc cgtgagcgca tgggacacat tgagctcgct gcaccagtaa			4586
90	cccacatttg gtacttcaag ggcgttccat cagcctcgg ctaccttttg gaccttgctc			4646

5 caaaggacct ggacctcatc atctacttcg gtgcgaacat catcaccagc gtggacgaag 4706
 aggctcgcca cagcgaccag accactcttg aggagaaat gcttctggag aagaaggacg 4766
 ttgaggcaga cgcagagtct gacattgctg agcgtgctga aaagctcgaa gaggatcttg 4826
 ctgaacttga ggcagctggc gctaaggccg acgctcgccg caaggttcag gctgctgccc 4886
 10 ataaggaaat gcagcacatc cgtgagcgtg cacagcgca aatcgatcgt ctcgatgagg 4946
 tctggcagac cttcatcaag cttgctccaa agcagatgat ccgcatgag aagctctacg 5006
 atgaactgat cgaccgctac gaggattact tcaccggtgg tatgggtgca gaggccattg 5066
 15 aggctttgat ccagaacttc gaccttgatg ctg 5099

20 <210> 6
 <211> 1165
 <212> PRT
 <213> Corynebacterium glutamicum

25 <400> 6
 Met Leu Glu Gly Pro Ile Leu Ala Val Ser Arg Gln Thr Lys Ser Val
 1 5 10 15
 Val Asp Ile Pro Gly Ala Pro Gln Arg Tyr Ser Phe Ala Lys Val Ser
 20 25 30
 30 Ala Pro Ile Glu Val Pro Gly Leu Leu Asp Leu Gln Leu Asp Ser Tyr
 35 40 45
 Ser Trp Leu Ile Gly Thr Pro Glu Trp Arg Ala Arg Gln Lys Glu Glu
 50 55 60
 Phe Gly Glu Gly Ala Arg Val Thr Ser Gly Leu Glu Asn Ile Leu Glu
 65 70 75 80
 40 Glu Leu Ser Pro Ile Gln Asp Tyr Ser Gly Asn Met Ser Leu Ser Leu
 85 90 95
 Ser Glu Pro Arg Phe Glu Asp Val Lys Asn Thr Ile Asp Glu Ala Lys
 100 105 110
 45 Glu Lys Asp Ile Asn Tyr Ala Ala Pro Leu Tyr Val Thr Ala Glu Phe
 115 120 125
 Val Asn Asn Thr Thr Gly Glu Ile Lys Ser Gln Thr Val Phe Ile Gly
 130 135 140
 Asp Phe Pro Met Met Thr Asp Lys Gly Thr Phe Ile Ile Asn Gly Thr
 145 150 155 160
 55 Glu Arg Val Val Val Ser Gln Leu Val Arg Ser Pro Gly Val Tyr Phe
 165 170 175
 Asp Gln Thr Ile Asp Lys Ser Thr Glu Arg Pro Leu His Ala Val Lys
 180 185 190

Val Ile Pro Ser Arg Gly Ala Trp Leu Glu Phe Asp Val Asp Lys Arg
 195 200 205
 5 Asp Ser Val Gly Val Arg Ile Asp Arg Lys Arg Arg Gln Pro Val Thr
 210 215 220
 Val Leu Leu Lys Ala Leu Gly Trp Thr Thr Glu Gln Ile Thr Glu Arg
 225 230 235 240
 10 Phe Gly Phe Ser Glu Ile Met Met Ser Thr Leu Glu Ser Asp Gly Val
 245 250 255
 Ala Asn Thr Asp Glu Ala Leu Leu Glu Ile Tyr Arg Lys Gln Arg Pro
 15 260 265 270
 Gly Glu Gln Pro Thr Arg Asp Leu Ala Gln Ser Leu Leu Asp Asn Ser
 275 280 285
 20 Phe Phe Arg Ala Lys Arg Tyr Asp Leu Ala Arg Val Gly Arg Tyr Lys
 290 295 300
 Ile Asn Arg Lys Leu Gly Leu Gly Gly Asp His Asp Gly Leu Met Thr
 305 310 315 320
 25 Leu Thr Glu Glu Asp Ile Ala Thr Thr Ile Glu Tyr Leu Val Arg Leu
 325 330 335
 His Ala Gly Glu Arg Val Met Thr Ser Pro Asn Gly Glu Glu Ile Pro
 30 340 345 350
 Val Glu Thr Asp Asp Ile Asp His Phe Gly Asn Arg Arg Leu Arg Thr
 355 360 365
 35 Val Gly Glu Leu Ile Gln Asn Gln Val Arg Val Gly Leu Ser Arg Met
 370 375 380
 Glu Arg Val Val Arg Glu Arg Met Thr Thr Gln Asp Ala Glu Ser Ile
 385 390 395 400
 40 Thr Pro Thr Ser Leu Ile Asn Val Arg Pro Val Ser Ala Ala Ile Arg
 405 410 415
 Glu Phe Phe Gly Thr Ser Gln Leu Ser Gln Phe Met Asp Gln Asn Asn
 45 420 425 430
 Ser Leu Ser Gly Leu Thr Tyr Lys Arg Arg Leu Ser Ala Leu Gly Pro
 435 440 445
 50 Gly Gly Leu Ser Arg Glu Arg Ala Gly Ile Glu Val Arg Asp Val His
 450 455 460
 Pro Ser His Tyr Gly Arg Met Cys Pro Ile Glu Thr Pro Glu Gly Pro
 465 470 475 480
 55 Asn Ile Gly Leu Ile Gly Ser Leu Ala Ser Tyr Ala Arg Val Asn Pro
 485 490 495
 Phe Gly Phe Ile Glu Thr Pro Tyr Arg Arg Ile Ile Asp Gly Lys Leu

	500	505	510
	Thr Asp Gln Ile Asp Tyr Leu	Thr Ala Asp Glu Glu	Asp Arg Phe Val
	515	520	525
5	Val Ala Gln Ala Asn Thr His Tyr Asp Glu Glu Gly Asn Ile Thr Asp		
	530	535	540
10	Glu Thr Val Thr Val Arg Leu Lys Asp Gly Asp Ile Ala Met Val Gly		
	545	550	555
	Arg Asn Ala Val Asp Tyr Met Asp Val Ser Pro Arg Gln Met Val Ser		
	565	570	575
15	Val Gly Thr Ala Met Ile Pro Phe Leu Glu His Asp Asp Ala Asn Arg		
	580	585	590
	Ala Leu Met Gly Ala Asn Met Gln Lys Gln Ala Val Pro Leu Ile Arg		
	595	600	605
20	Ala Glu Ala Pro Phe Val Gly Thr Gly Met Glu Gln Arg Ala Ala Tyr		
	610	615	620
	Asp Ala Gly Asp Leu Val Ile Thr Pro Val Ala Gly Val Val Glu Asn		
	625	630	635
25	Val Ser Ala Asp Phe Ile Thr Ile Met Ala Asp Asp Gly Lys Arg Glu		
	645	650	655
30	Thr Tyr Leu Leu Arg Lys Phe Gln Arg Thr Asn Gln Gly Thr Ser Tyr		
	660	665	670
	Asn Gln Lys Pro Leu Val Asn Leu Gly Glu Arg Val Glu Ala Gly Gln		
	675	680	685
35	Val Ile Ala Asp Gly Pro Gly Thr Phe Asn Gly Glu Met Ser Leu Gly		
	690	695	700
	Arg Asn Leu Leu Val Ala Phe Met Pro Trp Glu Gly His Asn Tyr Glu		
	705	710	715
40	Asp Ala Ile Ile Leu Asn Gln Asn Ile Val Glu Gln Asp Ile Leu Thr		
	725	730	735
45	Ser Ile His Ile Glu Glu His Glu Ile Asp Ala Arg Asp Thr Lys Leu		
	740	745	750
	Gly Ala Glu Glu Ile Thr Arg Asp Ile Pro Asn Val Ser Glu Glu Val		
	755	760	765
50	Leu Lys Asp Leu Asp Asp Arg Gly Ile Val Arg Ile Gly Ala Asp Val		
	770	775	780
	Arg Asp Gly Asp Ile Leu Val Gly Lys Val Thr Pro Lys Gly Glu Thr		
	785	790	795
55	Glu Leu Thr Pro Glu Glu Arg Leu Leu Arg Ala Ile Phe Gly Glu Lys		
	805	810	815

	Ala	Arg	Glu	Val	Arg	Asp	Thr	Ser	Met	Lys	Val	Pro	His	Gly	Glu	Thr	
				820					825					830			
5	Gly	Lys	Val	Ile	Gly	Val	Arg	His	Phe	Ser	Arg	Glu	Asp	Asp	Asp	Asp	
			835					840					845				
	Leu	Ala	Pro	Gly	Val	Asn	Glu	Met	Ile	Arg	Ile	Tyr	Val	Ala	Gln	Lys	
		850					855					860					
10	Arg	Lys	Ile	Gln	Asp	Gly	Asp	Lys	Leu	Ala	Gly	Arg	His	Gly	Asn	Lys	
	865					870					875					880	
	Gly	Val	Val	Gly	Lys	Ile	Leu	Pro	Gln	Glu	Asp	Met	Pro	Phe	Leu	Pro	
					885					890					895		
15	Asp	Gly	Thr	Pro	Val	Asp	Ile	Ile	Leu	Asn	Thr	His	Gly	Val	Pro	Arg	
				900					905					910			
20	Arg	Met	Asn	Ile	Gly	Gln	Val	Leu	Glu	Thr	His	Leu	Gly	Trp	Leu	Ala	
			915					920					925				
	Ser	Ala	Gly	Trp	Ser	Val	Asp	Pro	Glu	Asp	Pro	Glu	Asn	Ala	Glu	Leu	
		930					935					940					
25	Val	Lys	Thr	Leu	Pro	Ala	Asp	Leu	Leu	Glu	Val	Pro	Ala	Gly	Ser	Leu	
	945					950					955					960	
	Thr	Ala	Thr	Pro	Val	Phe	Asp	Gly	Ala	Ser	Asn	Glu	Glu	Leu	Ala	Gly	
					965					970					975		
30	Leu	Leu	Ala	Asn	Ser	Arg	Pro	Asn	Arg	Asp	Gly	Asp	Val	Met	Val	Asn	
				980					985					990			
	Ala	Asp	Gly	Lys	Ala	Thr	Leu	Ile	Asp	Gly	Arg	Ser	Gly	Glu	Pro	Tyr	
35			995				1000						1005				
	Pro	Tyr	Pro	Val	Ser	Ile	Gly	Tyr	Met	Tyr	Met	Leu	Lys	Leu	His	His	
		1010					1015					1020					
40	Leu	Val	Asp	Glu	Lys	Ile	His	Ala	Arg	Ser	Thr	Gly	Pro	Tyr	Ser	Met	
	025				1030						1035					1040	
	Ile	Thr	Gln	Gln	Pro	Leu	Gly	Gly	Lys	Ala	Gln	Phe	Gly	Gly	Gln	Arg	
				1045					1050						1055		
45	Phe	Gly	Glu	Met	Glu	Val	Trp	Ala	Met	Gln	Ala	Tyr	Gly	Ala	Ala	Tyr	
			1060					1065					1070				
	Thr	Leu	Gln	Glu	Leu	Leu	Thr	Ile	Lys	Ser	Asp	Asp	Val	Val	Gly	Arg	
50		1075					1080						1085				
	Val	Lys	Val	Tyr	Glu	Ala	Ile	Val	Lys	Gly	Glu	Asn	Ile	Pro	Asp	Pro	
		1090				1095						1100					
55	Gly	Ile	Pro	Glu	Ser	Phe	Lys	Val	Leu	Leu	Lys	Glu	Leu	Gln	Ser	Leu	
	105					1110				1115					1120		
	Cys	Leu	Asn	Val	Glu	Val	Leu	Ser	Ala	Asp	Gly	Thr	Pro	Met	Glu	Leu	
				1125					1130						1135		

Ala Gly Asp Asp Asp Asp Phe Asp Gln Ala Gly Ala Ser Leu Gly Ile
1140 1145 1150

5 Asn Leu Ser Arg Asp Glu Arg Ser Asp Ala Asp Thr Ala
1155 1160 1165

10 <210> 7
<211> 1775
<212> DNA
<213> Corynebacterium glutamicum

15 <220>
<221> CDS
<222> (500)..(880)
<223> rpsL gene

20 <400> 7
cagctctaca agagtgtcta agtggcgggc attccatgct ttggaggagc gatcttcaaa 60
ttcttccaaa gtgagttgac ctcgggaaac agctgcagaa agttcatcca cgacttggtt 120

25 tcggttaagg tcagtggcga gcttctttgc tggttcgttt ccttgaggaa cagtcattggg 180
aaccattcta acaagggatt tgggtgttttc tgcggctagc tgataatgtg aacggctgag 240
tcccactctt gtagttggga attgacggca cctcgactc aagcgcggtta tcgcccctgg 300

30 ttttccggga cgcggtggcg catgtttgca tttgatgagg ttgtccgtga catgtttggt 360
cgggcccaaa aaagagcccc cttttttgcg tgtctggaca ctttttcaaa tccttcgcca 420

35 tcgacaagct cagccttcgt gtctgtcccc cgggcgctcac gtcagcagtt aaagaacaac 480
tccgaaataa ggatggttc atg cca act att cag cag ctg gtc cgt aag ggc 532
Met Pro Thr Ile Gln Gln Leu Val Arg Lys Gly
1 5 10

40 cgc cac gat aag tcc gcc aag gtg gct acc gcg gca ctg aag ggt tcc 580
Arg His Asp Lys Ser Ala Lys Val Ala Thr Ala Ala Leu Lys Gly Ser
15 20 25

45 cct cag cgt cgt ggc gta tgc acc cgt gtg tac acc acc acc cct aag 628
Pro Gln Arg Arg Gly Val Cys Thr Arg Val Tyr Thr Thr Thr Pro Lys
30 35 40

50 aag cct aac tct gct ctt cgt aag gtc gct cgt gtg cgc ctt acc tcc 676
Lys Pro Asn Ser Ala Leu Arg Lys Val Ala Arg Val Arg Leu Thr Ser
45 50 55

55 ggc atc gag gtt tcc gct tac atc cct ggt gag ggc cac aac ctg cag 724
Gly Ile Glu Val Ser Ala Tyr Ile Pro Gly Glu Gly His Asn Leu Gln
60 65 70 75

gag cac tcc atg gtg ctc gtt cgc ggt ggt cgt gtt aag gac ctc cca 772
Glu His Ser Met Val Leu Val Arg Gly Gly Arg Val Lys Asp Leu Pro
80 85 90

```

      ggt gtc cgt tac aag atc gtc cgt ggc gca ctg gat acc cag ggt gtt      820
      Gly Val Arg Tyr Lys Ile Val Arg Gly Ala Leu Asp Thr Gln Gly Val
                    95                      100                      105

5      aag gac cgc aag cag gct cgt tcc ccg cta cgg cgc gaa gag ggg ata      868
      Lys Asp Arg Lys Gln Ala Arg Ser Pro Leu Arg Arg Glu Glu Gly Ile
                    110                      115                      120

10     att aaa aat gcg taaatcagca gctcctaagc gtccagtagt tcaggaccct      920
      Ile Lys Asn Ala
                    125

      gtatacaagt ccgagctcgt taccagctc gtaaacaaga tcctcatcgg tggcaagaag 980
15     tccaccgcag agcgcacgtc ctacgggtgca ctcgagatct gccgtgagaa gaccggcacc 1040
      gatccagtag gaaccctcga gaaggctctc ggcaacgtgc gtccagacct cgaagttcgt 1100
20     tcccgcctgt ttggtggcgc tacctaccag gtgccagtgg atgttcgccc agagcgcgca 1160
      aacaccctcg cactgcgttg gttggtaacc ttcaccctgc agcgtcgtga gaacaccatg 1220
25     atcgagcgtc ttgcaaacga acttctggat gcagccaacg gccttggcgc ttccgtgaag 1280
      cgtcgcgaag acaccacaaa gatggcagag gccaaccgcg ccttcgctca ctaccgctgg 1340
      tagtactgcc aagacatgaa agcccaatca cctttaagat caacgcctgc cggcgccctt 1400
30     cacatttgaa taagctggca gcctgcgttt cttcaaggcg actgggcttt tagtctcatt 1460
      aatgcagttc accgctgtaa gatagctaaa tagaaacact gtttcggcag tgtgttacta 1520
35     aaaaatccat gtcacttgcc tcgagcgtgc tgcttgaatc gcaagttagt ggcaaatgt 1580
      aacaagagaa ttatccgtag gtgacaaact ttttaatact tgggtatctg tcatggatac 1640
      cccggttaata aataagtga taccgtaac caacaagttg ggggtaccact gtggcacaag 1700
40     aagtgcctaa ggatctaaac aagggtccgca acatcggcat catggcgcac atcgatgctg 1760
      gtaagaccac gacca
                                     1775

45     <210> 8
      <211> 127
      <212> PRT
      <213> Corynebacterium glutamicum

50     <400> 8
      Met Pro Thr Ile Gln Gln Leu Val Arg Lys Gly Arg His Asp Lys Ser
          1                      5                      10                      15

55     Ala Lys Val Ala Thr Ala Ala Leu Lys Gly Ser Pro Gln Arg Arg Gly
          20                      25                      30

      Val Cys Thr Arg Val Tyr Thr Thr Thr Pro Lys Lys Pro Asn Ser Ala
          35                      40                      45

```

Leu Arg Lys Val Ala Arg Val Arg Leu Thr Ser Gly Ile Glu Val Ser
50 55 60

5 Ala Tyr Ile Pro Gly Glu Gly His Asn Leu Gln Glu His Ser Met Val
65 70 75 80

Leu Val Arg Gly Gly Arg Val Lys Asp Leu Pro Gly Val Arg Tyr Lys
85 90 95

10 Ile Val Arg Gly Ala Leu Asp Thr Gln Gly Val Lys Asp Arg Lys Gln
100 105 110

Ala Arg Ser Pro Leu Arg Arg Glu Glu Gly Ile Ile Lys Asn Ala
115 120 125

15

Patent claims

1. An isolated polynucleotide from coryneform bacteria,
containing a polynucleotide sequence coding for the
rpoB gene, selected from the group
 - 5 a) polynucleotide that is at least 70% identical with a
polynucleotide that codes for a polypeptide
containing the amino acid sequence of SEQ ID No. 2,
 - b) polynucleotide that codes for a polypeptide
containing an amino acid sequence that is at least
10 70% identical with the amino acid sequence of SEQ ID
No. 2,
 - c) polynucleotide that is complementary to the
polynucleotides of a) or b), and
 - 15 d) polynucleotide containing at least 15 consecutive
nucleotides of the polynucleotide sequence of a), b)
or c),

the polypeptide preferably exhibiting the activity of
the β -subunit of RNA polymerase B.
2. A polynucleotide as claimed in claim 1, wherein the
20 polynucleotide is a DNA, preferably a recombinant DNA,
that is replicatable in coryneform bacteria.
3. A polynucleotide as claimed in claim 1, wherein the
polynucleotide is an RNA.
4. A polynucleotide as claimed in claim 2, containing the
25 nucleic acid sequence as shown in SEQ ID No. 2.
5. A replicatable DNA as claimed in claim 2, containing
 - (i) the nucleotide sequence shown in SEQ ID No. 1, or

- (ii) at least one sequence that corresponds to sequence (i) within the region of the degeneracy of the genetic code, or
 - (iii) at least one sequence that hybridizes with the sequence that is complementary to sequence (i) or (ii), and optionally
 - (iv) sense mutations in (i) that are neutral in terms of function.
6. A replicatable DNA as claimed in claim 5, wherein the hybridization is carried out under a stringency corresponding to not more than 2x SSC.
7. A polynucleotide sequence as claimed in claim 1, which codes for a polypeptide containing the amino acid sequence shown in SEQ ID No. 2.
8. A coryneform bacteria in which the rpoB gene is enhanced, especially overexpressed.
9. A process for the production of L-amino acids, especially L-lysine, by fermentation, which process comprises carrying out the following steps:
- a) fermenting the coryneform bacteria that produce the desired L-amino acid, in which bacteria at least the rpoB gene or nucleotide sequences coding therefor are enhanced, especially overexpressed;
 - b) concentrating the L-amino acid in the medium or in the cells of the bacteria, and
 - c) isolating the L-amino acid.
10. A process as claimed in claim 9, wherein there are used bacteria in which further genes of the biosynthesis pathway of the desired L-amino acid are additionally enhanced.

11. A process as claimed in claim 9, wherein there are used bacteria in which at least some of the metabolic pathways that reduce formation of the desired L-amino acid are excluded.
- 5 12. A process as claimed in claim 9, wherein there is used a strain transformed using a plasmid vector, and the plasmid vector carries the nucleotide sequence coding for the rpoB gene.
- 10 13. A process as claimed in claim 9, wherein expression of the polynucleotide(s) coding for the rpoB gene is enhanced, especially overexpressed.
14. A process as claimed in claim 9, wherein the regulatory/catalytic properties of the polypeptide (enzyme protein) for which the polynucleotide rpoB codes are increased.
- 15 15. A process as claimed in claim 9, wherein for the production of L-amino acids there are fermented coryneform microorganisms in which at the same time one or more genes selected from the group
- 20 15.1 the gene dapA coding for dihydrodipicolinate synthase,
- 15.2 the gene gap coding for glyceraldehyde 3-phosphate dehydrogenase,
- 15.3 the gene tpi coding for triose phosphate isomerase,
- 25 15.4 the gene pgk coding for 3-phosphoglycerate kinase,
- 15.5 the gene zwf coding for glucose-6-phosphate dehydrogenase,
- 30 15.6 the gene pyc coding for pyruvate carboxylase,

- 15.7 the gene mgo coding for malate quinone oxidoreductase,
- 15.8 the gene lysC coding for a feed-back resistant aspartate kinase,
- 5 15.9 the gene lysE coding for lysine export,
- 15.10 the gene zwal coding for the Zwal protein
- 15.11 the gene rpsL coding for ribosomal protein S12
- are enhanced or overexpressed.
- 10 16. A process as claimed in claim 9, wherein for the production of L-amino acids there are fermented coryneform microorganisms in which at the same time one or more genes selected from the group
- 16.1 the gene pck coding for phosphoenol pyruvate carboxykinase,
- 15 16.2 the gene pgi coding for glucose-6-phosphate isomerase,
- 16.3 the gene poxB coding for pyruvate oxidase
- 16.4 the gene zwa2 coding for the Zwa2 protein
- are attenuated.
- 20 17. A coryneform bacteria containing a vector that carries a polynucleotide as claimed in claim 1.
18. A process as claimed in one or more of the preceding claims, wherein microorganisms of the species *Corynebacterium glutamicum* are used.
- 25 19. A method of finding RNA, cDNA and DNA, in order to isolate nucleic acids, or polynucleotides or genes, that code for the β -subunit of RNA polymerase B or are

very similar to the sequence of the rpoB gene, which method comprises using as hybridization probes the polynucleotide containing the polynucleotide sequences as claimed in claims 1, 2, 3 or 4.

- 5 20. A method as claimed in claim 18, wherein arrays, micro arrays or DNA-chips are used.
21. A DNA from coryneform bacteria, coding for β -subunits of RNA polymerase B, wherein the associated amino acid sequences have been altered between positions 1 to 10
10 in SEQ ID No. 2 by amino acid substitution.
22. A DNA as claimed in claim 21, which DNA codes for β -subunits of RNA polymerase B, the associated amino acid sequences containing at position 5 in SEQ ID No. 2 L-leucine, L-isoleucine or L-valine.
- 15 23. A DNA from coryneform bacteria, coding for β -subunits of RNA polymerase B, wherein the associated amino acid sequences have been altered between positions 190 to 200 in SEQ ID No. 2 by amino acid substitution.
- 20 24. A DNA as claimed in claim 23, which DNA codes for β -subunits of RNA polymerase B, the associated amino acid sequences containing at position 196 in SEQ ID No. 2 L-phenylalanine or L-tyrosine.
- 25 25. A DNA from coryneform bacteria, coding for β -subunits of RNA polymerase B, wherein the associated amino acid sequences have been altered between positions 420 to 450 in SEQ ID No. 2 by amino acid substitution.
- 30 26. A DNA as claimed in claim 25, which DNA codes for the β -subunits of RNA polymerase B, the associated amino acid sequences containing at positions (sic) 439 in SEQ ID No. 2 any proteinogenic amino acid with the exception of L-histidine.

27. A DNA as claimed in claim 25, which DNA codes for the β -subunits of RNA polymerase B, the associated amino acid sequences containing at position 424 in SEQ ID No. 2 L-proline or L-arginine.
- 5 28. A DNA as claimed in claim 25, which DNA codes for the β -subunits of RNA polymerase B, the associated amino acid sequences containing at position 425 in SEQ ID No. (sic) L-threonine or L-alanine.
- 10 29. A DNA as claimed in claim 25, which DNA codes for the β -subunits of RNA polymerase B, the associated amino acid sequences containing at position 426 in SEQ ID No. 2 L-leucine or L-lysine.
- 15 30. A DNA as claimed in claim 25, which DNA codes for the β -subunits of RNA polymerase B, the associated amino acid sequences containing at position 429 in SEQ ID No. (sic) L-isoleucine, L-valine or L-leucine.
- 20 31. A DNA as claimed in claim 25, which DNA codes for the β -subunits of RNA polymerase B, the associated amino acid sequences containing at position 444 in SEQ ID No. 2 L-leucine, L-tyrosine or L-tryptophan.
32. A DNA as claimed in claim 25, which DNA codes for the β -subunits of RNA polymerase B, the associated amino acid sequences containing at position 446 in SEQ ID No. 2 L-proline or L-isoleucine.
- 25 33. A DNA from coryneform bacteria, coding for β -subunits of RNA polymerase B, wherein the associated amino acid sequences have been altered simultaneously at one or more positions selected from the group a) position 1 to 10, b) position 190 to 200 and c) position 420 to 450 in SEQ ID No. 2 by amino acid substitution.
- 30 34. A DNA as claimed in claim 33, which DNA codes for the β -subunits of RNA polymerase B, the associated amino

acid sequences containing in SEQ ID No. 2 at one or more positions selected from the group a) position 5 L-leucine, b) position 196 L-phenylalanine, c) position 429 L-valine and d) position 439 L-tyrosine.

- 5 35. A DNA as claimed in claim 34, which DNA codes for the β -subunit of RNA polymerase B, the associated amino acid sequence containing L-leucine at position 5, L-phenylalanine at position 196 and L-valine at position 429, shown in SEQ ID No. 4.
- 10 36. A DNA as claimed in claim 34, which DNA codes for the β -subunit of RNA polymerase B, the associated amino acid sequence containing L-tyrosine at position 439, shown in SEQ ID No. 6.
- 15 37. A DNA from coryneform bacteria, coding for the β -subunit of RNA polymerase B, wherein the base sequence of the DNA contains thymine at position 715, thymine at position 1288 and thymine at position 1987, shown in SEQ ID No. 3.
- 20 38. A DNA from coryneform bacteria, coding for the β -subunit of RNA polymerase B, wherein the base sequence of the DNA contains thymine at position 2016, shown in SEQ ID No. 5.

Abstract

The invention relates to an isolated polynucleotide containing a polynucleotide sequence selected from the group

- 5 a) polynucleotide that is at least 70% identical with a polynucleotide that codes for a polypeptide containing the amino acid sequence of SEQ ID No. 2,
- b) polynucleotide that codes for a polypeptide containing
10 an amino acid sequence that is at least 70% identical with the amino acid sequence of SEQ ID No. 2,
- c) polynucleotide that is complementary to the polynucleotides of a) or b), and
- d) polynucleotide containing at least 15 consecutive
15 nucleotides of the polynucleotide sequence of a), b) or c),

and to a process for the production of L-amino acids by fermentation using coryneform bacteria in which at least the rpoB gene is present in enhanced form, and to the use of polynucleotides containing the sequences of the invention as hybridization probes.